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THE HISTOGENESIS OF BONE MARROW

A MORPHOLOGICAL AND CYTOLOGICAL STUDY OF MYELOGENESIS.

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Introduction.

The observation recorded in this thesis complete with a previous work, a study of the processes of cell growth and change, which occur during the development and formation of mammalian bone, and bone marrow.

Chondrogenesis and osteogenesis have been shown to be manifestatious of different growth impulses arising in the same tissue.

In this present work it is intended to trace the stages of a further growth activity in this tissue, of an entirely different order; the process of myelogenesis.

Although recognised as the most important of the blood cell factories, the bone marrow in its developmental stages has received little attention from the multitude of workers on haematology. This is the more remarkable when the divergent views on the cytogenetic relationships of blood cells and marrow cells are considered. It is in these problems that haematologists have failed to find agreement and that difficulties/

difficulties have arisen to sustain endless controversies, into which there is no need to enter; further no useful purpose would be served, since this work aims simply at recording a series of definite observations.

The processes whereby living cells undergo developmental changes, both morphological and functional are represented in fixed preparations by phases which only when correlated indicate the path along which development was proceeding at the moment of death. This is constantly to be borne in mind in the study of haemopoiesis where the morphological values alter so completely in cells very closely related.

In order to render the statement of the changes that occur in various cells, concisely and clearly, and incidently to avoid the involved nomenclature employed in haematological literature, no terms will be used to denote cells which have been interrupted during a specialised maturation. No benefit is derived from such a proceedure, and indeed the mere fact of interposing a name, tends to falsify the conception of a process, the essential feature of which/

which is uninterrupted development.

When a mesénchyme cell changes into an osteoblast many transition phases can be demonstrated with fixed preparations none of which are stable even to the extent of hesitating to undergo mitosis. In the formation of blood cells, both in the white and red blood cell series, transition forms derived from the stem blood cell appear to act otherwise. Proliferation of the transition forms constitutes one of the most striking features in both series, moreover in the early stages of development of these blood cells, stable forms arise which may proceed with maturation at any time, or on the other hand senility and death may supervene. One stem blood cell may by division of its derivatives give rise to an unlimited number of blood cells, the complete evolution of which by virtue of the hesitation and division of transition forms may be spread over the entire existence of the organism, the initial change in the stem blood cell having occurred in embryonic life. Fully developed marrow consists mainly of the hesitating transition forms and their derivitives. The stem blood cells, never numerous in the sense that the transition forms are numerous even in early embryonić life, are not a prevalent type in full grown marrow. Sometimes not one can be demonstrated in an entire field. For these/

these reasons it is abundantly clear that the total changes that occur in the evolution of the blood cells may never be encountered in preparations of marrow itself. The illustrations and descriptions encountered in this work, were made from early embryonic marrow either in the first stages of its evolution or in a zone where the tissue was in process of generation.

Of previous investigators the results although obtained with a different technique correspond more closely with those of Maximow (IX) than any other observer. Many of the conclusions are not in accordance with much that has hitherto had current acceptance. To keep within the limits of a simple record these issues will not be raised; to do so would involve a reduntant survey of the literature, an interminable discussion on technique and the un-:ravelling of an unwieldy terminology which has passed beyond the limits of scientific application. In this last respect it is sufficiently significant to record that Grüner (V) in his glossary of haematological terms has in one case, eighty odd synonyms for a single cell. It is the special aim of this work to describe the morphological values of the cell entities involved in myelogenesis, and define their genetic relationships.

The Endothelium.

Under the above heading it is proposed to trace the formation of the capillary endothelium of the blood vessels of bone and bone marrow in order to establish the relationship of the cell of this tissue with the other specialised connective tissue elements that also develop. The extension by proliferation of the osteogenic mesenchyme into the capsules of the hypertrophic cartilage cells, both in the diaphysis and the epiphysis has previously been described(X) In the diaphysis it was shown to extend in two separate zones towards the extremities moving at a rate commensurate with the preparatory changes in the cartilage. These cartilaginous changes register the amount of growth in length of the diaphysis and the invading tissue was shown to consolidate the framework of chondromucin, by the formation of osteoblasts and the deposition of ossein. The two zones of tissue forming the ossification lines, consisting of syncytial masses of actively dividing indifferent connective tissue cells, Fig. I and II, ceaselessly repeat the processes of special cell formation throughout the whole growth period, depositing in their wake the/

the products of their cytogenetic activity. The development of osteoblasts and osteoclasts has al:ready been described. The development of the endothelial cells has also been lightly surveyed but in view of the fact that these cells retain the essential morphological characters of the indifferent mesenchyme cells and that other genetic activities have been ascribed to them by other workers it is perhaps expedient to discuss their origin in greater detail.

The earliest sign of capillary formation is observed in the syncytial masses invading the hypertrophic cartilage zone. This formation continues with the advance of the mesenchyme at the ossification lines and may be studied there at any stage in bone growth. Transverse sections made at the ossification line, through the mesenchyme tissue, show in the plasm of the syncytium apparent vacuoles, Figs. III and IV, the larger ones occasionally contain red blood corpuscles but in this region the content is usually coagulated fluid. Traced in serial section they are seen to be distinct tubes continuous centrally with formed capillaries. The lumina appear to enlarge at the expense of the syncytial cells, the nuclei of which are pushed aside until they ultimately form the walls of/

of lumina, Fig. V. No change in structure of these nucleii occur other than a gradual flattening. Fig.VI. In this way the endothelial cells arise in situ from the mesenchyme. At no stage in their development are they discrete, nor have they been observed to play any further role either of division or separation. Even in later stages where the adjacent syncytial cells separate to form a reticulum of stroma cells no evidence of further differention has been discovered. It would appear that the mechanism of capillary formation is associated with fluid pressure of the general circulation, which exerts a boring force into the plasma of the syncytium. Whether this be so or not it is abundantly clear that, the endothelial cells are formed without specialisation and segregation and that proximity to the lumina that arise, decides their subsequent function as cells of a limiting membrane to the blood stream. As the adjacent mesenchyme cells loosen during the isolation and mobilisation of the osteoblasts and stem blood cells, the capillaries came to lie in or near the centre of the intertrabecular areas, Fig. V. Later the lumina increase in size and sinusoid blood spaces are formed, without which the marrow tissue developes, Fig. X.XI.

Still/

Still later occasional small arteries are formed, the indifferent stroma cells aggregating by division to form the plain muscle fibres.

The Development of the Stem Blood Cell

The use of the term stem blood cell is admittedly questionable, for not only does it give rise to the antecedents of the blood cells, but also it is the parent cell of the megakaryocytes. However, these lines of development appear to be the limits of its cytogenetic potential in physiological processes of growth, so no confusion can arise with regard to its present application.

This cell forms by a direct maturation of indifferent mesenchyme cells in a somewhat similar manner to the osteoblast. The later cells, however, develope first and it is only when the mesenchyme has delivered its quota of osteoblasts to the chondromucin trabeculae that the stem blood cell begins to make its appearance, Fig. V. They develop in the extravascular mesenchyme, Fig. VI, eventually freeing themselves, and in this way the remaining indifferent mesenchyme cells assume the form of a reticulum, the stem blood cells having mobilised themselves freely in the interstices of a meshwork.

Thus/

Thus a zone of reticular tissue is formed in the wake of the syncytial mesenchyme in which capillaries occupy the central positions and epithelial like layers of osteoblasts line the calcified chondromucin trabeculae, Fig.VIII & X, occupying the intervals between the capillaries and the bone forming cells, the still indifferent mesenchyme cells now sparsely scattered from a loose reticulum giving support to the capillaries and enfolding in a network the stem blood cells. The subsequent fate of these reticular cells will be considered later. The point of inter:est at this stage is that the stem blood cell is the predominant feature of the histological picture. This reticular zone is always present, immediately central to the syncytium at the ossification line and affords every oportunity for the study of the stem blood cell before it moves along the paths of further development. Its identity can here be established without qualification because its derivations have not yet arisen to confuse its genetic kinship, moreover by virtue of its priority of development both in time and manner, it definitely establishes its precedence in relation to the other myeloid/

myeloid elements. In no other part of the developing bone is it so prevalent, and even in this area it is not as numerous as would be expected, when the myriads of cells which arise from it are estimated. Its development has been described as a process of maturation of an indifferent mesenchyme cell. During its evolution no division has been observed in any phase. The cytomorphosis is characteristic and peculiar to the cell, especially the changes that occur in the nucleus. From the pale vesicular nucleus of the mesenchyme cell, Fig.III, a deeply basophil nucleus arises, somewhat larger in size. The chromatin increases in amount and aggregates to form a large purplish, violet-staining chromatin body, irregular in shape and indifferent in its position. Figs. XIV, XV, XVI. Sometimes it lies near the nuclear membrane and occasionally it appears to be fused with it, in which case it exerts an influence on the nuclear membrane so that the nucleus appears to be partly folded on itself. Occasionally it is seen as two, three, or four partially separated masses connected by means of stout chromatic strands. Other small granules of chromatin are usually seen, studded throughout the nucleus, especially around the periphery of the nuclear membrane. Changes in the cytoplasm develop pari passu with the nuclear metamorphosis, the most striking being the intense basophilia/

basophilia. This is apparent before the cell is isolated. The final mobilisation is associated with a general contraction of the cell body, so that when finally rounded off it lies freely in a fluid space, (Figs. V and VII,) surrounded by the still indifferent connective tissue cells which are now rapidly assum:ing the reticular formation.

The mature stem blood cell is highly amoeboid, hence in sections its size and shape are liable to assume wide variations, Fig. XIV. Occasionally small club shaped pseudopodia project from the entire circumference of the cell.

Similar processes are seen in megakaryocytes both in smear and bulk preparation, and are supposed, by some observers, (Wright (XII), Woodcock(XI) and others), to be associated with the formation of blood platelets.

It is non-granular inasmuchas it possesses no special granules. The fact that it may or may not contain azur granules is unimportant, as these are not specific to any cell of the blood series. In the mature cell there is little difference in the staining reaction of nucleus and cytoplasm, both are intensely basophilic. Senile types however show a diminished intensity/

intensity of basophilia. Advanced senile types show a greatly swollen nucleus and are orthochrommatic in staining reaction. These large pale degenerating cells die in karyorrhexis Fig. (LVIII). Protean in form, Figs, XIV, XV. XVI. actively amoeboid, capable of ageing rapidly, such gross morphological factors as size, shape and staining reaction, are valueless in establishing the identity of the cell, in fact any cell whose functions are associated with such wide activities. The characteristic structure of the nucleus, and the absence of special granules in an almost homogeneous cytoplasm, are the features which distinguish the stem blood cell from all other types. Romanowsky's stain and its modifications such as Jenner's and Leishman's, fail in the demonstration of its structure, and for this reason alone, are of little value in haematological cytology.

A well defined attraction sphere makes its appearance during the development of the cell, showing up as a pale area, by contrast during the basophil changes in the cytoplasm, Fig. XIV. It lies close into the nuclear membrane, which is always indented at this site. Neither the indentation of the nucleus or the attraction sphere, are seen unless orientated in the cell in the plane of section. It is/

is apparent that the different appearances assumed by the cell according to the above description offer no just grounds for inferring that definite morphological variants occur. Nor is there, as far as has been ascertained, any reason to suppose as the dualists do, that because of the subsequent divergent cytogenetic activities there must be structural differences. The fact that a fertilised ovum is totipotent for every cell in the organism is sufficient to permit the assumption that the stem blood cell is multipotent for all cells of blood and bone marrow. It is of interest to record that no appearances have been observed that would lead to the supposition that the stem blood cells arise from the endothelium. Although they may be found in close proximity to the capillary walls, on no occasion have they been observed within the lumina, nor have endothelial cells been seen tending to changes, that the mesenchyme cell undergoes, during its maturation into a stem blood cell.

The Genesis of Red Blood Cells.

The most prevalent process not only in myelogenesis but also in fully developed marrow, is the development of red blood corpuscles.

Although numerous accounts are given in the literature, unanimity has not been attained, and detailed work is singularly lacking on the earliest cells of the series. The tissue undergoing haemopoiesis, central to the reticular zone (already referred to in the previous paragraph), affords the most favourable site for this study because of the loose arrangement of the cell elements. Fig.X to XIII. Myelogenesis proceeds so rapidly - a single stem blood cell giving rise to hundreds of derivatives within a very short period of growth - that oblique sections are necessary to expose the greatest area of the intertrabecular spaces, a procedure which has the effect of apparently slowing down the process by extending the zone of transition and at the same time rendering for observation the maximum number of cells. In such a section, the great numerical increase in the myeloid elements is shown to occur in focal areas which arise in (Fig. IX), relation to the/

the scattered stem blood cells.

The stem blood cells form the nidus of the haemopoietic foci , all gradations occuring from single cells through small isolated clusters up to large focal areas which eventually coalesce, Figs. XII and XIII. This focal arrangement is most characteristic of the early embryonic marrow and is the result of extensive proliferation in the descendants of the parent cells. It is impossible to decide from fixed preparations, whether a stem blood cell can maturate directly into a fully formed erythocyte, (heteroplastic development). The power of division evidenced by mitotic figures in the various derivatives is indicative of homoplastic development whereby, the terminal stages alone undergo simple maturation without proliferation. Certainly the stem blood cells are so few that heteroplastic development would not account for more than a tithe of the myriads of myeloid elements that comprise the developed marrow tissue. The outstanding feature of erythropoiesis is the extreme cytomorphosis that occurs. Whether the changes take place during division, or whether advance in the series is made by the resting forms, is not easy to decide. Such issues can only be settled experimentally/

experimentally with living preparations. As in the stem blood cell senile changes may arise in any stage of development. The fact indicates a reserve of developmental potential, which has been amply demonstrated clinically, whereby urgent physiological demands and pathological interruptions can be rapidly met.

Structural changes in the nucleus of the stem blood cell indicate that the path of red cell development has been entered.

The chromatin mass fragments and bars or nodules of chromatin scatter themselves irregularly throughout the nucleoplasm, Fig. XVI. At the same time there is a slight reduction in the size of the cell mass, Fig.XVII. Though still amoeboid these cells are usually round or oval in shape and the nucleus usually occupies a central position, Figs XVIII & XX. Further changes in the chromatin disposition ensue, which are seen in aggregations around the nuclear membrane, Figs. XVIII and XIX. Later these aggregations form irregular bars which project towards the centre, Fig.XIX. During these changes no alteration in the cytoplasm occurs, other than a reduction in amount./

Cells of the red series at this stage bear no resemblance to any other cell types. They are non granular and the chromatin arrangement is specific. The subsequent cytomorphosis is associated with the further changes in the nucleus, the chromatin bars assuming the radial arrangement which has evoked the description of "wheel nucleus". Alternating bars of parachromatin and chromatin passing from the membrane to the centre, give this appearance when the cell is cut through its greatest diameter. Whilst these nuclear changes are taking place the cytoplasm becomes less basophil and more homogeneous, the reticulum of the spongioplasm becoming less evident. Ultimately the cytoplasm is orthochromatic and then gradually as the haemoglobin content rises, becomes definitely acidophil. The further changes resulting in the formation of the small nucleated red cell are well known. With the reduction in size of the nucleus the chromatin bars become more closely approximated, and the parachromatin is no longer apparent. The fate of the nucleus is a problem still freely dis-:puted.

Karyorrhexis, extrusion, disintegration and solution have all in turn been put forward as possible terminations/

terminations. Maximow (/X.), is in favour of extrusion. Frequently the nucleus assumes an eccentric position in the cell body, so that an ex-:tensive zone of nuclear membrane is thinly bounded by cytoplasm, Fig.XXVIII. Sections made through the plane of this eccentric nuclear protrusion give the appearance of a marked nucleus, Fig.XVIII. Further extreme eccentricity may be taken as evidence of impending extrusion. However, these appearances are inadequate evidence for the assumption of such a fate especially as they are only occasional.

The prevalent appearances have led to the conclusion that disintegration and solution following pycnotic degeneration is the fate of the nucleus. The dense staining chromatin mass becomes reduced in size and gradually loses its basophilia, the membrane crenates and eventually, an indistinct slightly basophil irregular structure is all that remains. Occasionally the Cabot ring formation is seen in the last stages, Fig.XXXII, which are apparently very rapid once the degenerating chromatin loses its basophil reaction, Fig.XXVIII to XXXII.

When the stem blood cell embarks on the development of the red series the structural modifications described, definitely separate cells of this group from all other types. The expediency of introducing terms/

terms to define stages in the evolution of the erythrocyte is questionable, for by so doing stress is laid on the more characteristic transition phases which in reality are no more significant in the process than those left unnamed.

The way in which the red blood cells gain the circulation is amply illustrated in the early stages of myelogenesis, that is by passage through the endothelium. Although no light can be thrown on the phenomenon, it ~~possibly~~ illustrates the plasticity of the endothelial cytoplasm, a property already illustrated in the plasm of the mesenchyme syncytium in capillary formation. Erythrocytes are the first mature cell to generate in bone marrow although certain forms of leucocytes arising by heteroplastic development appear to arise almost simultaneously. The bulk of these cells nevertheless make their appearance at a later stage as do the megakaryocytes and fat cells.

The Granulocytes.

Under this heading the development of the cells with specific granules which maturate into blood leucocytes will be considered. The specific granules vary in character chemically and morphologically in different animals and are usually referred to in terms of their staining reactions. In rabbits and guineapigs the three types are, the special granules or pseudo-eosinophil, true eosinophils, and basophil or mast cell granules. The first named are the most numerous and ~~not~~ probably are homologous with the neutrophil granules in man. The question of the specificity of these granules as definite structural entities has been settled elsewhere. Downey's (1V) work on guinea pigs has been verified by the methods that he employed using Ehrlich's three colour stain, eosin, indulin, and aurantia. With the technique employed in this work it was not possible to demonstrate the mast cell granules in rabbits owing to their extreme solubility in the watery fixative used. The mast cell granules in guinea pigs are not soluble to the same extent and are clearly demonstrable, especially if the tissue is immersed in a decalcifying solution such/

such as Perenyi. The nitric acid increasing their affinity for basophil dyes.

Maximow (/X.) has dealt exhaustively with the development of these cells, and it is mainly with the idea of confirmation that the processes are here set forth in detail although one or two issues such as a common primitive granular form for eosinophil and pseudoeosinophil has not been sustained. This point will be again raised in dealing with the atypical early true eosinophil granules. In order to avoid repetition it is proposed to describe the special granular cell development in detail and then later refer to the other types, only inasmuch as points of special interest arise.

The first granular cells to appear, arise in the central fringe of the reticular zone, but they are so few that they are apt to escape observation. Attention was first directed to them by the discovery of a cluster numbering five, lying in the reticular zone amidst a group of stem blood cells and undifferentiated reticular cells. They were fully matured polymorph leucocytes of the eosinophil type, with highly refractile coarse rod shaped granules. Further in-:vestigation/

investigation revealed, very occasionally, these mature types, both of the eosinophil and special granular cell series occurring in the same zone. Earlier forms were also encountered and this has led to the assumption that they arise rapidly by heteroplastic maturation directly from the stem blood cells, a form of development which is subsequently super:ceded by homoplastic evolution. Dantschakoff(11) has described a similar phenomenon in birds and, Maximow (18) records the same thing in rabbits and guinea pigs. The homoplastic development occurs first of all, in the focal zones of erythropoiesis, where the special granular cells appear singly most frequently, Fig.XXXV, in pairs occasionally, and in groups of three and four very rarely. They lie extravascularly amidst the other tissue elements. No special type of stem blood cell can be associated with the granulocyte path of development. The granules at first appear slowly, so that it is not unusual to find a cell with all the appearances of a stem blood cell showing one, two, three or four granules, Fig.XXXVI., lying around the outer periphery of the attraction sphere. At this stage no structural changes take place in the nucleus nor is the general staining/

staining reaction of the cell altered. The presence of the granules is the sole indication that a new line of development has been started. These special granules stain a deep red, are round or slightly oval in shape, varying very little in size, the smaller ones are apparently the most recently developed. Increase in the number of granules marks the further development of the cell, division occuring at any stage. Maximow puts forward the hypothesis that granulation first occurs during the last stages of mitosis in a stem blood cell. In support of this he says that early granular cells are never seen in the early mitotic figures; that is in prophase, but always in telophase. Hence he decides that granules appear at the end of karyokinesis itself, and that therefore the qualitative changes connected with this line of differentiation are intimately bound up with the process of mitotic division. The finding of single cells quite isolated showing as few as one to four granules, renders this point of view untenable; nor does the same theory appear to be applicable to the initial stages of erythopoiesis on the same grounds.

With regard to the megakaryocyte, Maximow admits that/

that in this case it is difficult to decide when a stem blood cell enters the path of megokarycyte development. It will be shown later that this cell maturates, without division interrupting the process. The arrangement of the granules appears to occur around the attraction sphere in the form of a cone. With the use of panchromatic plates and Wratten filters the granules can be cut out and the position of the sphere indicated in relation to the disposition of the granules, Fig.XXXIX and XL. The further changes that occur after a full complement of granules develop, are seen in staining reactions and nuclear form and structure. Plate LII. The early granulocytes are large cells, if anything slightly larger than a stem blood cell, but thereafter the subsequent changes are associated with a gradual reduction in size until the polymorph stage is reached.

The cytoplasm of the early typesis deeply baso:phil, but towards the end of granulation it gradually becomes orthochromatic tending to acidophilia, Plate LII. This change also occurs first of all in the vicinity of the attraction sphere, gradually extend:ing/

extending throughout the cell plasm., Fig. XLII. The chromatin mass of the nucleus also becomes paler, and at the same time appears to contract forming a rounded body, Figs.XLI and XLII. The changes in the shape of the nucleus are well known, passing throughout the various forms which have merited recognition by numerous appellations. Again these terms fail to assist in any understanding of the process, because they sharply define types which are variable and in constant fluctuation. Certainly the nucleus is at one time reniform, another sausage shaped, then lobulated and finally polymorphous, but in living tissue the transition is continuous. At the reniform stage the chromatin body fragments and forms smaller chromatin masses which eventually appear as nucleolar-like structures, one in each lobulation of the mature nucleus. Plate III.

After the nucleus lobulates, division is not longer possible, and for the most part it is only the early types which divide. During the whole process of the formation of special granular leucocytes, no change occurs in the nature of the granules. They are/

are simply endogenous bodies arising from the cyto-:plasm as a result of metabolic changes.

In intra-uterine life in rabbits, very few mature special leucocytes arise. The homoplastic method of development is a slow process and although the granu-locytes appear very early, the mature forms are not developed in large numbers until the marrow is well advanced.

In rabbits the true eosinophil granulocytes arise in a similar way to the special granulocytes, but in this type the granules themselves are atypical in the early stages, but at the same time quite distinctive. Maximow is inclined to the belief that in embryonic marrow there is a common granular cell form, for both eosinophils and special granular cells, although he admits that later in development the two groups have separate origins. The technique here employed by no means suggests such an interpretation.

The early forms of the true eosinophil granulocytes develop granules of a larger size with quite different staining reaction, varying in colour from a pale violet to a bright red. Downey (IV) has demonstra-ted/

demonstrated that the basophil quota in these cells form the immature granules and that maturation is associated with increased acidophilia. Certainly these cells are rare in early myelogenesis and for that reason are difficult to study, but the granules are quite specific even in the earliest stage. Later the granules become more highly refractile and appear to lie in small rounded pale areas of cytoplasm. Eventually they elongate and become rod shaped, some of them straight others slightly curved. With re:gard to the mast leucocytes there is little to add to what has hitherto been said regarding the other types. A technique is still wanted which will demonstrate them in bulk tissue in rabbits in such a way that they stand comparison with the other granulocytes, in cytological detail. In guinea pigs they develop in late foetal life and are prevalent constituents of marrow in young animals. The granules stain a deep purplish violet with the polychrome methylene blue and are much larger than the eosinophils and special granules. In every way they evince the same changes in development which have been described for the special granulocyte. In embryonic myelogenesis/

myelogenesis in guinea pigs, the mast cell of histogenic type is freely encountered. Towards the end of foetal life it disappears. It bears no relation to the haematogenous type which forms the mast leucocyte.

Smear preparations fixed in osmic acid vapour and stained with Mallory's triple connective tissue stain afford a very satisfactory technique for the study of this cell. There is nothing to add to what is already known regarding the nature of this cell in guinea pigs. Downey's work has been confirmed both with his own technique and the present method.

The Magakaryocyte.

The origin of the megakaryocyte is more easily observed than any other of the myeloid elements hence it is difficult to conceive how opposed statements have arisen. Jordon (VII) reviews the literature and deals with the various origins and functions ascribed to this cell. The work of Wright perhaps has attracted in this connection more attention than that of most observers. He derives the megakaryocyte from the endothelium, a point of view not upheld, and regards it as the source of blood platelets supported by Bunting(I), Jordan(VII), Woodcock(XI) and others. This appears to be an attractive hypothesis but is yet to be proved. Certainly these cells are peculiar to mammalian haemopoietic foci, occurring in the embryonic liver, spleen and bone marrow. That they may have some philogenetic relation to the thrombocytes of the lower vertebrates, has yet to be established. In the compact marrow they are frequently seen lying close to the endothelial sinuses, with club shaped psendopodia bulging into the lumen and also into the adjacent myeloid elements.

elements. It is suggested by the above observers, because of similarity in staining reactions, that these protoplasm buds separate to form the blood platelets. It has been shown above that the stem blood cell develops in certain amoeboid phases similar psendopodia, but in neither of these cells has the separation of these protoplasmic projections been observed. Phagocytosis and erythopoiesis are functions that have been ascribed to the megakaryocyte, neither of which have been observed during the course of this investigation. Jordon's megakaryocyte is not the same cell originally classified by Howell but is a precursor . Howell's megakaryocyte is the cell referred to in this work, since it has not been possible to verify Jordon's classification either on a genetic or functional basis. My observations on this cell, once again agree closely with those of Maximow, who refrains from committal on the question of function.

The stem blood cell moves along the line of megakaryocyte development without any initial structural changes to indicate the impending cytomorphosis, Fig.XLV and XLVI. Plate IV. At first it simply grows/

grows in size, both the cell body and nucleus enlarging proportionally. The nucleus then becomes increasingly indented forming either two or three ovoid lobes, Figs.XLV, 41, 42. At the same time the chromatin material increases in amount and aggregates in irregular masses. Further lobulations develop, some of which appear to separate, but always attenuated threads of nuclear membrane suspend them to the main nuclear mass.

Maximow and others regarded this lobulation as a "multipolar amitosis". From the material investigated there appears to be no reason to suggest any specific process other than that which occurs in the maturation of a polymorph leucocyte nucleus; except that the process is repeated a large number of times in various segments of the nuclear body, so that the resultant form of the nucleus is elaborately protean, consisting merely of a complex system of sessile lobulations which vary in size and arrangement. Such terms as annular, horse-shoe, crescentic, labate, may be applicable to the same nucleus, depending on the plane of section; the composite form being so variable that it defies description in simple analogy, Fig.XL to XLIII./

XLIII. Nucleolar-like bodies of chromatin appear in each lobulation, together with other angular masses of chromatin on the linin network. The cytoplasm is deeply basophil, increasing in amount with the development of the nucleus. The three zones described by Carnegie Dickson (111) have not been seen. These cells arise early in myelogenesis, but not until erythopoiesis and granulopoiesis have made considerable progress. They may be readily found in long bones of rabbit embryos of eighteen days.

They usually first develop in groups of two or three and at this stage show no special preference for the walls of the capillary sinusoids. It is only in later embryonic life, when they develop in larger numbers, that they appear to be frequently related to the vessel walls. Large pale orthochromatic degenerating forms are met with in all stages of development. Megakaryocytes have no genetic or functional connection with the polykaryocytes or osteoblasts. Figs.XII & LV.

The Phagocytes of Bone Marrow

The subject of these cells from the pathological standpoint is a difficult and involved problem which cannot be entered upon, although it is felt that certain light can be shed, from the study of these cells in normal growing marrow, on the genesis and function of many such cells found in aberrant growth conditions of marrow, such as occur in pernicious anaemia.

All the phagocytes found were cells of the undifferentiated mesenchyme type, not arising however from the endothelium although of the same morphological value, but developing from the reticular and stroma cells in situ; merely by segregation and isolation, following the ingestion of particles of nuclear debris or red

blood corpuscles. In the early stages of this work, certain large cells were isolated in smear preparations, containing within the cytoplasm numerous haemoglobin staining bodies, from two to thirty odd, varying in size from a small, barely visible globule to that of an erythrocyte. These preparations were obtained from/

from the marrow near the ossification line, of newly born rabbits and guinea pigs.

Although with the technique employed, (Jenner's) it was impossible to classify the cells, it was thought that they were special erythropoietic cells forming R.B.C's endogenously as plastids. This view was further strengthened by the fact that at this stage other cell debris was not found in the cytoplasm of these cells. Later however, apparently similar cells were seen with chromatin particles interspersed with the haemoglobin globules. These were usually smaller and although they may have been cells of a different type, the original point of view was definitely threatened . With the adoption of new methods, these cells were all demonstrated to have the same morphological value, and at the same time their genetic relationship with the undifferentiated mesenchyme cell was finally established.

In developing marrow these phagocytes are not very numerous. They are seen as discrete elements, Figs. LVI and LVII, Plate II. Frequently they contain chromatin bodies resembling the nucleus of the late red series. This fact has been adduced as additional/

additional evidence in favour of the extrusion theory in the final maturation of the red blood cell. However, these cells are very occasional in erythropoietic foci. They have a random distribution and the ingested material may be derived from any of the tissue elements, from chromatin to eosinophil granules. Haemaglobin is not infrequently present in these cells at the same time as the chromatin masses, which resemble the pycnotic nuclei. It would appear that they effect the removal of cell debris, especially senile types of myeloid cells which have hesitated in maturation and died.

The phagocytic cells found crowded with haemoglobin globules in newly born animals represent an extreme physiological destruction of red blood corpuscles, most possibly associated with respiratory changes following birth.

Fat Cells of Bone Marrow.

During the early stages of myelogenesis the sparsely arranged mesenchyme cells of the reticular zone become widely separated, forming a loose stroma almost obscured by the abundant profusion of the rapidly growing myeloid tissue.

In this stage, although flattened and elongated, the reticular cells still preserve the pale vesicular nucleus of indifferent mesenchyme. Fig.XXXIII & XXXIV.

As soon as extrauterine life begins the process of myelogenesis becomes less rapid and the cell elements, as a result, appear less crowded. Fat storage begins with this diminished haemopoietic activity and progresses until eventually the myeloid cells are forced into the ~~the~~ intersticies of an adipose stroma, which occupies the bulk of the marrow spaces.

The formation of fat cells from the reticular cells is associated with changes that precede the appearance of the fat globules. The flattening disappears, the cytoplasm increases in amount, and the/

the nucleus becomes larger and rounded. Eventually these cells become quite discrete and globules of fat appear first of all as minute granules which increase in bulk to form large globules. The nucleus then becomes eccentric in the cytoplasm and with the subsequent coalescence of the fat globules, finally flattens against the cell membrane.

During these processes of fat storage, no change occurs in the structure of the nucleus. As with the endothelial cells and phagocytes; it retains the characteristic nucleus of an indifferent undifferentiated mesenchyme cell, Fig. LIX.
(Preparations vide technique).

Summary.

Myelogenesis is a growth manifestation of the multipotency of the indifferent mesenchyme cell.

The cell changes which initiate the process, begin after the formation of osteoblasts and osteoclasts, the stem blood cell maturating from the same type of primitive connective tissue energid that gives rise to the cells of bone tissue.

It develops near the ossification line in the syncytium of mesenchyme cells finally freeing itself from this tissue by mobilisation of its cytoplasm.

Endothelial cells also arise from the mesenchyme syncytium in relation to channels which penetrate the syncytial plasm. They undergo no essential alteration in structure, retaining the vesicular nucleus of the indifferent mesenchyme cell. At no time are they isolated as free cells. Flattening is associated with fluid distension of the lumina which they bound. Following the development of capillaries and the formation and segregation of the stem blood cells the remaining cells of the syncytium assume the form of a reticulum in/

in the interstices of which the stem blood cells proceeds, with further development and proliferation, to form the myeloid elements.

The reticular cells form an attenuated stroma supporting the developing marrow cells. Some of them become free to act as phagocytes, others form plain muscle fibres along the walls of capillaries, thus converting them into small arteries. The fat cells also arise from the cells of the reticulum.

Both phagocytic and fat cells retain the vesicular form of nucleus that characterises the mesenchyme cell.

In haematogenesis the stem blood cell manifests three divergent lines of development:

(a) The red blood cell series by homoplastic development.

(b) The white blood cell series by both heteroplastic and homoplastic development, though the former is rapidly superceded by the latter.

(c) Megakaryocyte series, only by heteroplastic development.

In/

In the red blood cell and white blood cell series the early indication of these lines of development are characteristic and specific.

Changes in the structure of the nucleus of the stem blood cell, whereby the chromatin body fragments, indicates that the path of red cell evolution has been entered. The appearance of specific granules in the cytoplasm, without nuclear change in the stem blood cell, is indicative of the leucocyte line of develop-:ment. The specific granules of the granulocytes are endogenously formed. In rabbits and guinea pigs they are of three types. Small, round, acidophil: large, rod shaped acidophil, and basophil. In the granulocytes the nucleus maturates to form the poly-morph structure characteristic of the leucocyte.

In the red blood cell series, final maturation of the erythrocyte is associated with pycnotic degen-:eration of the nucleus and solution.

Extensive proliferation of the early types of both blood series occurs, creating a reserve for un-:limited demands and forming the bulk of myeloid cells in developed marrow.

Conclusion.

The simple interpretation of the facts recorded in this work renders many of the arduously disputed problems in haematogenesis superfluous. For this reason little use has been made of the literature, and the current teachings in haematology, especially in regard to the complex pedigrees of myeloid cells. It would appear that there are certain morphological features which are valueless in undertaking a classification of blood elements with a view to establishing genetic connections.

When a cartilage cell passes to the hypertropic stage from the resting stage, extreme differences in size and shape occur, and the staining reaction is modified throughout the process: changes, which occur
profoundly alter the gross histological character of the cell, although not affecting its essential structural features, and which are indisputably bound up with functional activities in the life cycle of the cell/

cell itself. Such morphological variations are manifested by various marrow cells, and if considered in assessing the types and orders of transition forms, lead to endless difficulties and unwarranted complications.

It is felt that the use of terms has tended to accentuate these fallacies and even for this reason alone it was deemed desirable to avoid the use of them. However applicable they may appear to be in fixed preparations, once applied to intermediate forms in a series, they have the effect of destroying cytogenetic values.

In the red blood cell series such terms as erythroblast and megaloblast, are currently used with the idea of defining stages, whilst in reality numerous transitional forms exist under these terms merging indefinitely one into the other, changing and proliferating ceaselessly towards the fulfilment of a final maturation, a process which cannot be interrupted even for the application of a mere term.

From the above points of view many of the controversies in haematogenesis lose their importance, especially those relating to the stem blood cell and its/

its multipotency. Maximow has ably thrashed out the issues involved in this dispute and it is sufficient to record that the facts elicited in this investiga-:tion support his argument, as far as myelogenesis is concerned.

TECHNIQUE.

Materials and Methods: Rabbit, and guinea pig were used exclusively. No essential differences in the processes described were found in these animals. Rabbits and guinea pigs form more convenient material than other animals, both because of the ease of breeding, and because of the small bulk of the tissue. The former are pre-eminently more suitable, foetal growth being extremely rapid, a gestation period of thirty days resulting in a full-time foetal animal, approximately the same size and weight as a full-time foetal guinea pig, which has a gestation period of seventy days.

All types of cartilage bones were used in various stages of development, especially the short and long bones of the fore and hind limbs. Metacarpal and metatarsal bones are exceptionally satisfactory -small, rapidly penetrated by fixatives: once the integument is removed, they require no further handling.

For purposes of description and illustration rabbits and guinea pigs were used.

The foetal animals were delivered through the abdominal/

abdominal wall, after killing the mother by fracture-dislocation of the neck. Tissue required was rapidly dissected and transferred to the fixative in as recent a state as possible.

<u>Fixation</u>: Bulk tissue, consisting of bones freed from the surrounding skin and muscle, was fixed in a saturated solution of mercuric chloride in saline, at 37° centigrade, to 100cc. of which ½ to 1½c.cs. of 1% osmic acid solution was added, immediately after introducing the tissue. To prevent vapourisation of the osmic acid, the specimen bottles were tightly corked. Long bones of more than 5mms. in diameter were sectioned longitudinally. Ten to seventeen hours immersion, depending on the size of the tissue, gives adequate fixation. If the quantity of osmic acid solution introduced is insufficient, the tissue be-:comes dark brown in colour. If left for a longer period, the mercuric chloride tends to make the tissue very brittle.

<u>Decalcification</u>: Foetal bones: rabbits up to the termination of the gestation period, were trans-:ferred after fixation ~~in~~ Zenker's fluid, for forty-eight hours. This treatment serves a double purpose, both decalcifying young bones, and acting as a satis-factory mordant, especially if azur stains are subse-:quently used. To preserve the fat cells, the tissue was immersed for eight to ten days in Zenker's fluid, with/ to?

with 2cc., 1% osmic acid to every 100cc of fixative.

Perenyi's solution was found to be the most suit:able reagent for the decalcification of more mature bone. If not used too long, the cell preservation is not affected, a small amount of shrinking obscures the finer detail, but does not interfere with the identification of the different cell elements.

<u>Teased and Smear Preparations</u>: These were treat:ed with the above fixative for ten minutes, and then transferred to Zenker's fluid for a further ten minutes. Osmic acid vapour was also used for smear preparations, in which case Mallory's triple connective tissue stain was found to give very satisfactory results.

<u>Embedding</u>: Paraffin and celloidin were both employed. For paraffin embedding, the tissue was passed through graduated spirits, beginning at 30%, and rapidly ascending through 50%, 60%, and 70% to 80%, hardening in the latter for eight to twelve hours, finally ascending to absolute through 90% alcohol, using two changes of the former over a period of four hours.

Cedar wood oil was used in two changes over 72 hours, for removing the alcohol.

The/

The tissue was then transferred direct to paraffin with a melting point either of 49° or 50° centigrade. Indifferent results may be anticipated if the paraffin is permitted to pass above 56°. Four baths of paraffin over twelve hours remove the cedar wood oil.

The thin sections obtained with the paraffin afford a considerable advantage over the celloidin method.

Staining: Eosin and azur, or Wright's (VIII) polychrome methylene blue, and eosin, give good results. The least capricious staining was obtained with 5% aqueous eosin and Grubler's polychrome methylene blue ¼%, differentiated in 3% colophonium in 95% alcohol (after Wright). This stain is especially good for cytological work when employed after fixation described above.

Illustrations.

The photographs were taken with a Leitz vertical camera and a Zeiss $\frac{1}{12}$ objective, oil immersions being used throughout. Wratten and Wainwright's pancromatic plates were employed in combination with Wratten filters. Coloured drawings were made from actual fields and added to the illustrations in order to show the colour values obtained with the technique. The ar:rangement conforms to the text, the object being to render a photographic representation of the processes described. All illustrations have been made from section preparations.

Fig. I. Proliferating syncytial mesenchyme at the ossification line erupting into the capsules of the dead hypertrophic cartilage cells.
(Oblique section).

Fig. II. Ditto. (transverse section).

Fig. III. Syncytial mesenchyme × 1800.

Fig. IV. Vacuolation of mesenchyme in capillary formation.

Fig. V. Later stage than IV shewing capillary formation and loosening of the cells during special maturation. A formed stem blood cell in the middle of the field.

Fig. VI./

Fig. VI. Capillary formation showing flattening of the endothelium and three stages in the development of a stem blood cell, (A,B,C,). A fully developed mobilised stem blood cell is lying freely in a fluid space (C).

Fig. VII. The same field × 1800, showing stages in the mobilisation of the cytoplasm from the general syncytial mass in the stem blood cell maturation.

Fig. VIII. Osteoblasts (A), and stem blood cells (B) in the reticular zone.

Fig. IX. Early myelogenesis in the reticular zone showing the extravacular activity of the stem blood cells.

Fig. X. An intertrabecular space showing central capillary, adjacent myeloid cells and osteoblsts de-:positing bone.

Fig. XI. XII. XIII. Extravascular myelogenesis in various stages. XII shows an osteoclast on a spicule of bone.

Fig. XIV. Three stem blood cells (A), showing variations in shape, size and staining reaction. The one on the extreme right shows partial separation of the chromatin mass. The two central ones show the indentation of the nucleus and the pale attraction sphere.

Figs/.

Fig. XV and XVI. Stem blood cells (A) in the reticular zone 1800, Early cells of the red series (B), and reticular cells (C).

Fig. XVII to XXXII. Erythropoiesis.

Fig. XVII. Showing the fragmentation of the chromatin mass (A).

Fig. XVIII. A group of cells (B), in the early red series, showing specific arrangement of the chrom-:atin round the nuclear membrane

Fig. XIX. Ditto.

Fig. XX to XXII. Showing phases in the early cells of red series with dividing forms (A).

Fig. XXIII. Showing the extravascular arrange-:ment in an erythropoietic focus.

Fig. XXIV. Showing changes in the cytoplasm during the development of haemoglobin (G and H Wratten Filters). A,B,C.

Fig. XXV. Showing the formation of the wheel nucleus. (D and G Wratten filters) A,B,C.

Fig. XXVI and XXVII. Showing condensation of the chromatin in the formation of the small nucleated red cells, A,B,C.

Fig. XXVIII. Showing various positions of the nucleus in the late red cell series. Some are defin-:itely eccentric. A.B.C.

Fig./

Fig. XXIX, XXX and XXXI. Pycnotic nuclei de-:generation and loosing basophilia (A).

Fig. XXXI. shows all stages from the stem blood cell to the fully developed erythrocytes. B.

Fig. XXXII. Cabot ring formation in the degen-:eration of the nucleus (C).

Fig. XXXIII. Erythropoietic focus showing retic-:ular cells, (B).

Fig. XXXIV. Reticular cells lying between two erythropoietic foci. (A stem blood cell in mitosis ?). It might possibly be a cell of the early red series.

Figs. XXXV to XLIV. Granulopoiesis.

Fig. XXXV. A field showing one early granulocyte (G).

Fig. XXXVI. An early psendo-eosinophil showing three developed granules around the periphery of the attraction sphere (K).

Fig. XXXVII. Early granulation showing the chromatin mass in the nucleus (K).

Fig. XXXVIII. A stem blood cell (A), and two early granulocytes (one in mitosis B.)

Fig. XXXIX. Granulocyte showing cone of granules (G and H filters).

Fig. XL. The same, all showing attraction sphere (D and G filters).

Fig./

Fig. XLI. Granulocyte showing condensation of the chromatin mass, K.

Fig. XLII. Granulocytes showing diminished baso-:philia in the region of the attraction sphere, (G and H filters).

Fig. XLIII. A group of four early granulocytes.

Fig. XLIV. An early granulocyte showing indenta-:tion of nucleus. Apart from the presence of the granules tis cell has the same morphological value as a stem blood cell.

Figs. XLV to LIV. The development of the megakaryocyte.

Fig. XLV. Stem blood cells 1800.

Fig. XLVI. Bilobulation.

Fig. XLVII. Milobulation.

Fig. XLVIII. Multilobulation showing the arrange-:ment of the chromatin.

Fig. XLIX and L. Early annular forms.

Fig. LI and LII. Young megakaryocytes showing apparent separation of nuclear masses.

Fig. LIII. A fully developed megakaryocyte adjacent to the wall of a sinusoid.

Fig. LIV. A megakaryocyte in the midst of a haemopoietic focus.

Fig. LV. A poly-karyocyte L 1800. A stem blood cell is seen at the top of the field (M).

Figs/.

Figs. LVI to LVIII. Phagocytes. (P).

Fig. LVII. A phagocyte (P), lying in a cluster of nucleated red cells containing ingested haemoglobin and nuclear debris.

Fig. LVIII. A phagocyte (P) with ingested chrom:atin material. A stem blood cell is seen in karyorr:hexis.

Fig. LIX. All stages in the evolution of a fat cell from undifferentiated mesenchyme cells (A). The three cells (B) show minute globules.

<u>Plate</u> I. Actual field of an erythropoietic focus, showing all transition forms, from the stem blood cell to the erythrocyte. An early granulocyte is seen (left). An early megakaryocyte, and undifferentiated reticular cells.

<u>Plate</u> II. Erythropoietic focus showing all forms, early granulocytes and a phagocyte with ingested haemo:globin and chromatin.

<u>Plate</u> III. Granulopoiesis drawn from different fields of the same section, showing various forms, from a stem blood cell to the mature leucocyte. The changes both in nuclear form and in the cytoplasm are seen.

<u>Plate</u> IV. Stages in the maturation of a mega-karyocyte from the stem blood cell, drawn from differ:ent fields of the same section.

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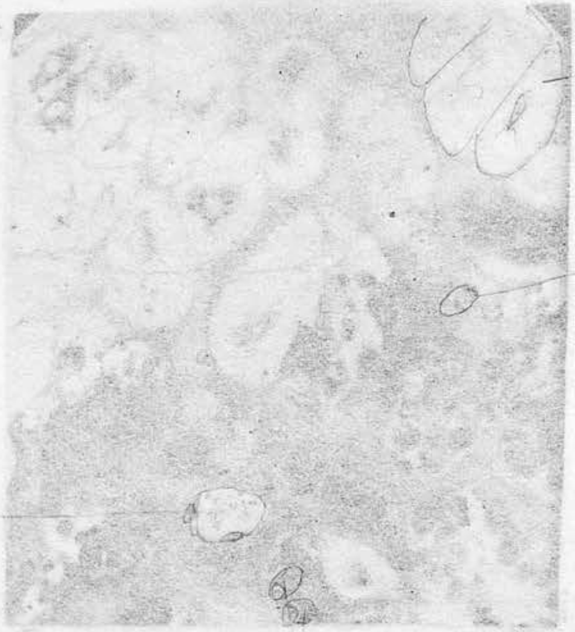


Dead hypertrophic cartilage cells

mesenchyme cell of syncytium in mitosis

young
ndothelium

osteoblasts

Fig II

Osteoblast

Fig III

Fig. I

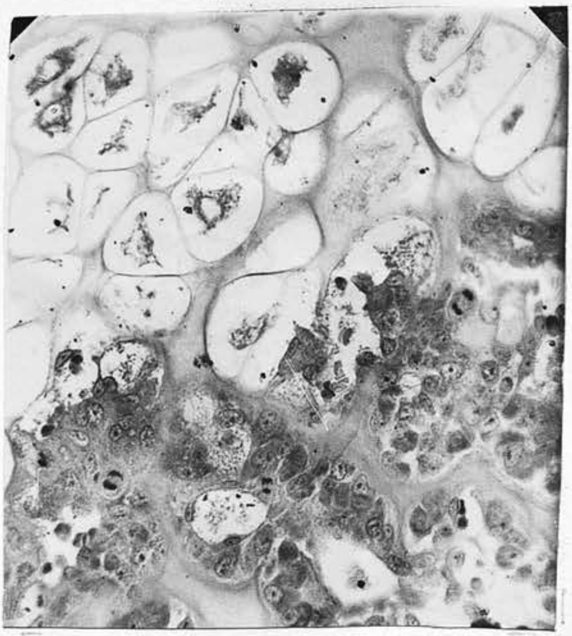


Fig II

Fig III

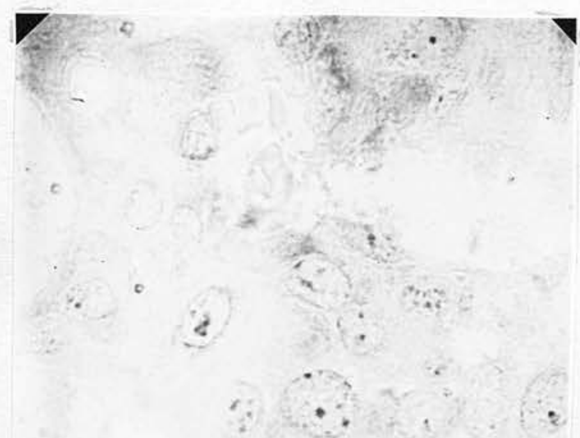
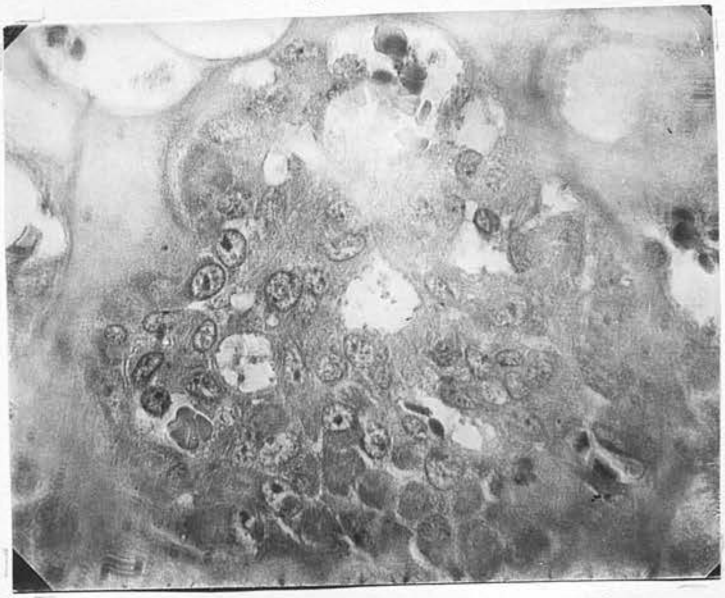


Fig IV

vacuolation of the mesenchyme syncytium in capillary formation

Fig V

Fig IV



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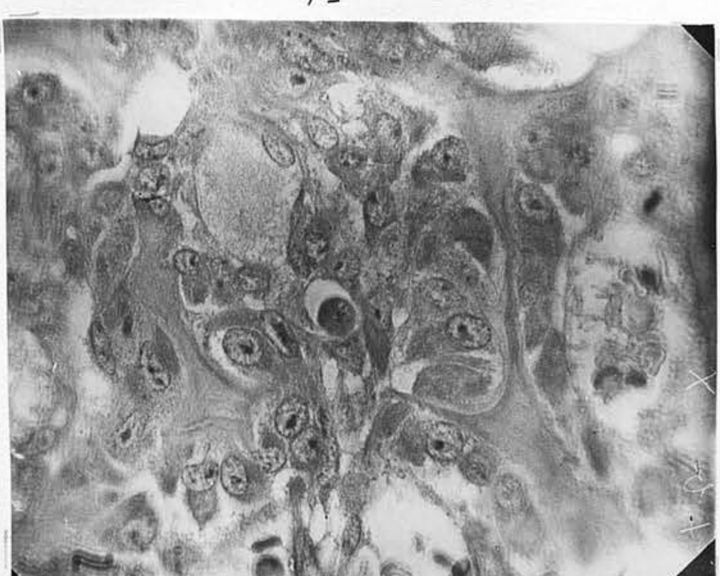

Fig. VII

Fig VI

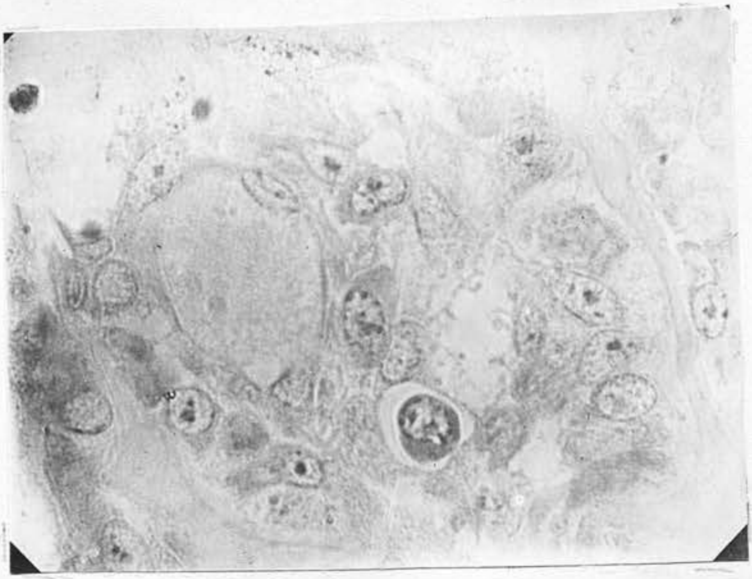

Fig. VII.

Fig VIII

Fig. IX

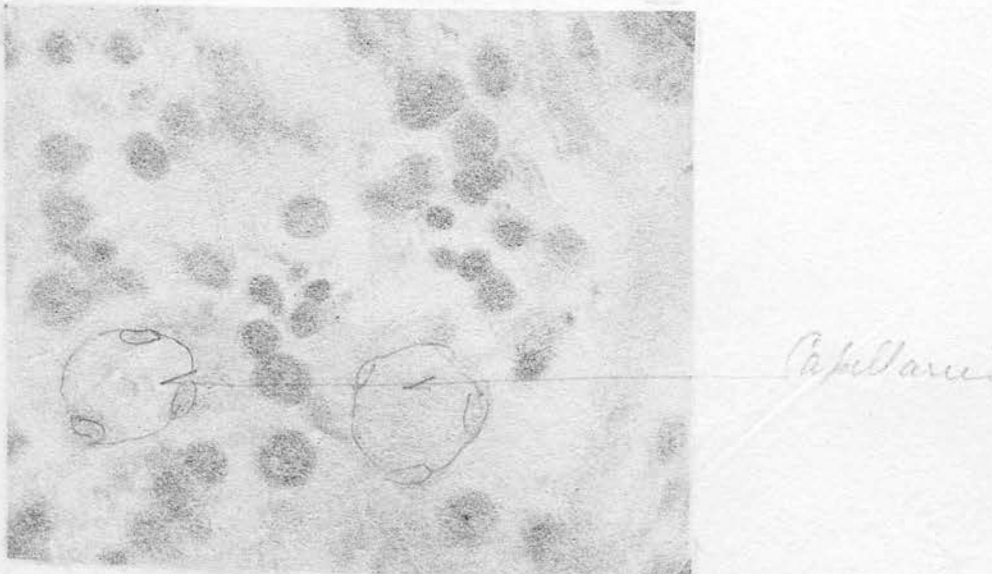

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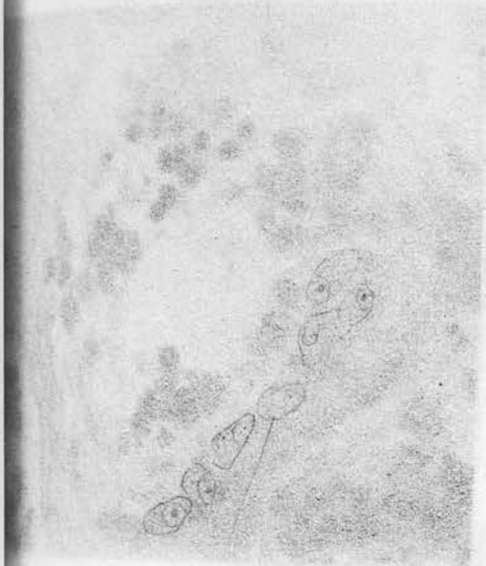
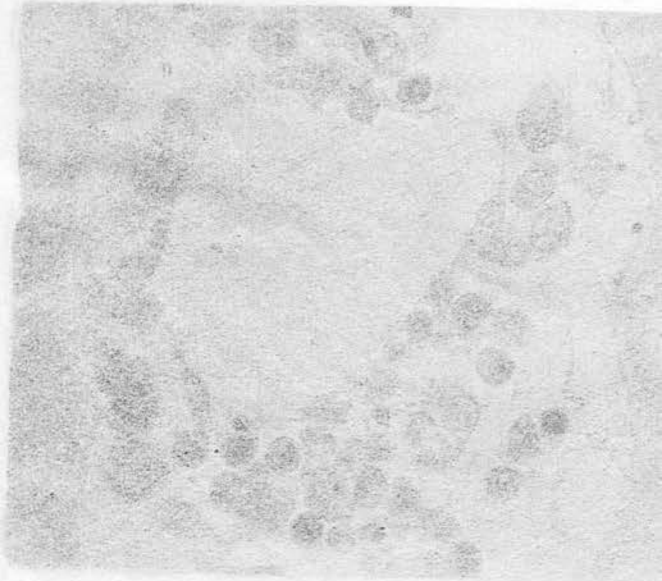
Fig. IX

Fig X



Osteoblasts

Fig XI



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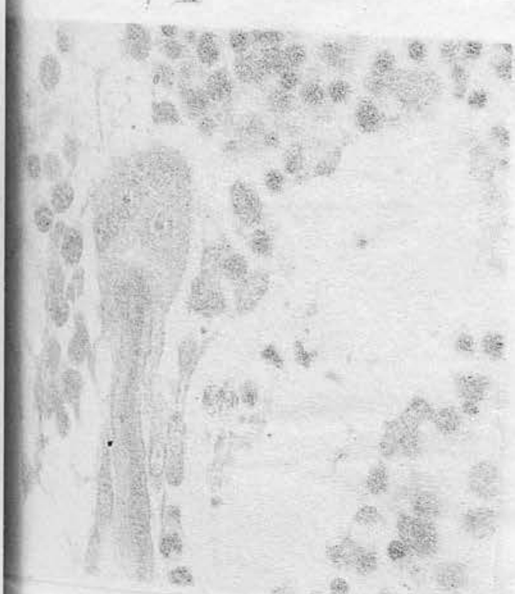

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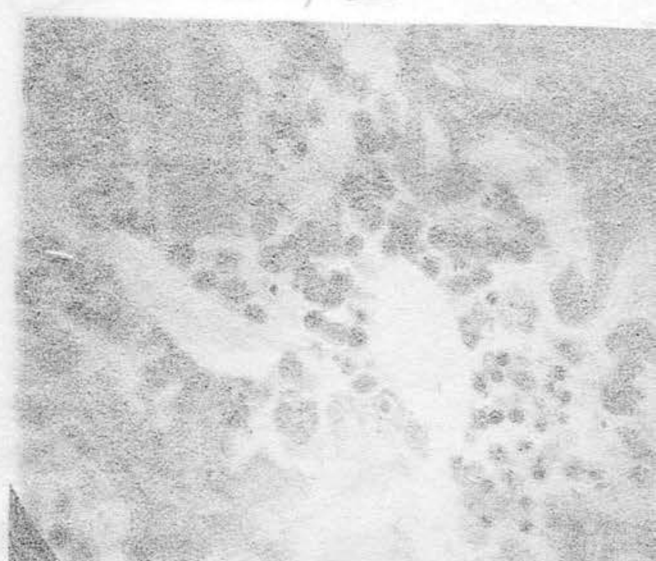

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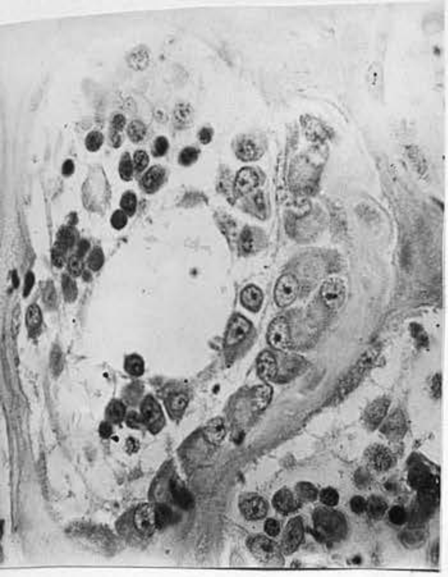

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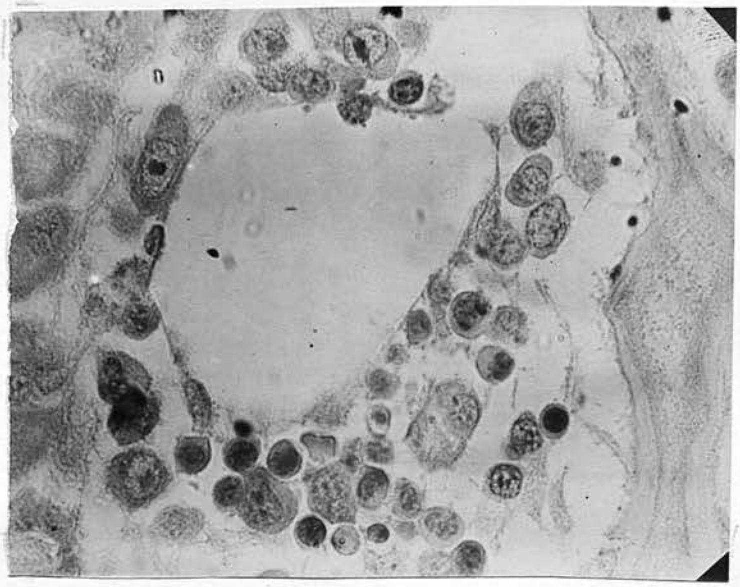

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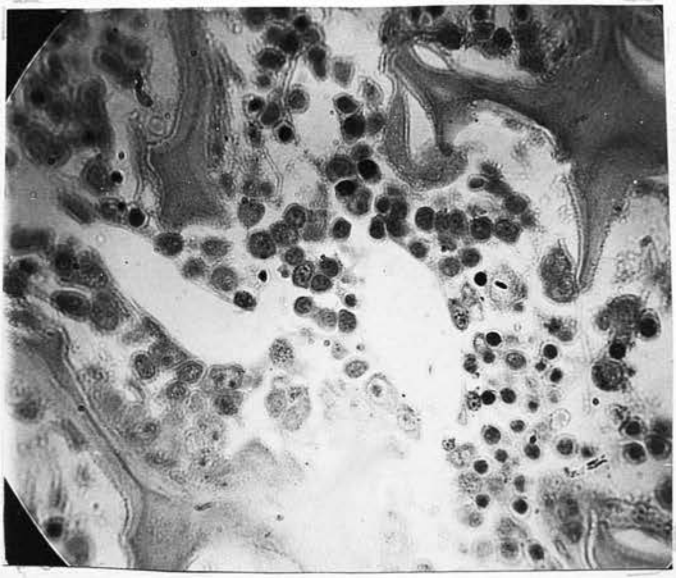
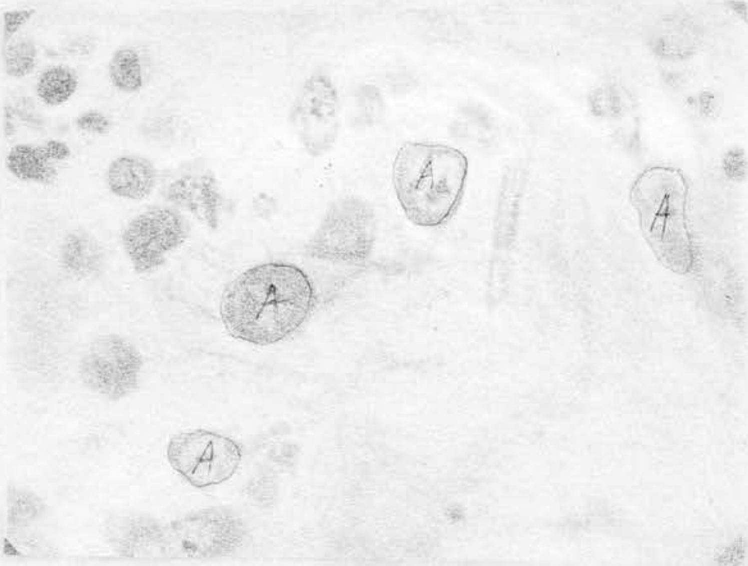
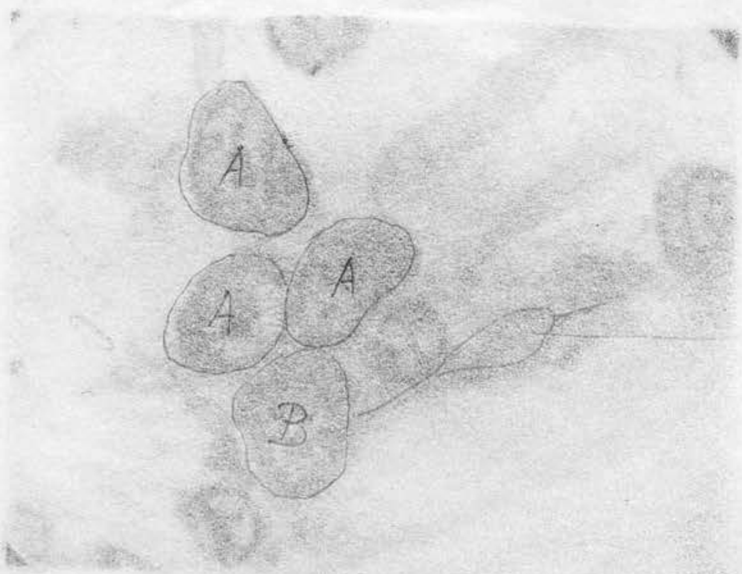
Fig XIII



Figures XIV XV XVI





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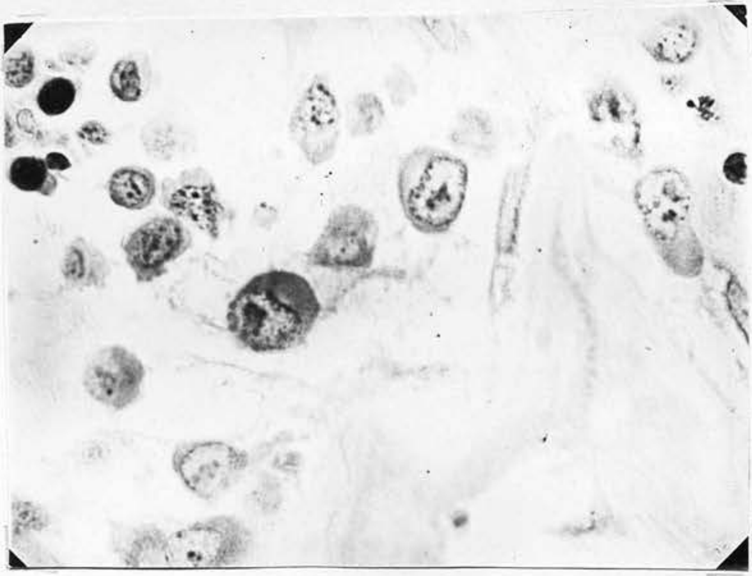

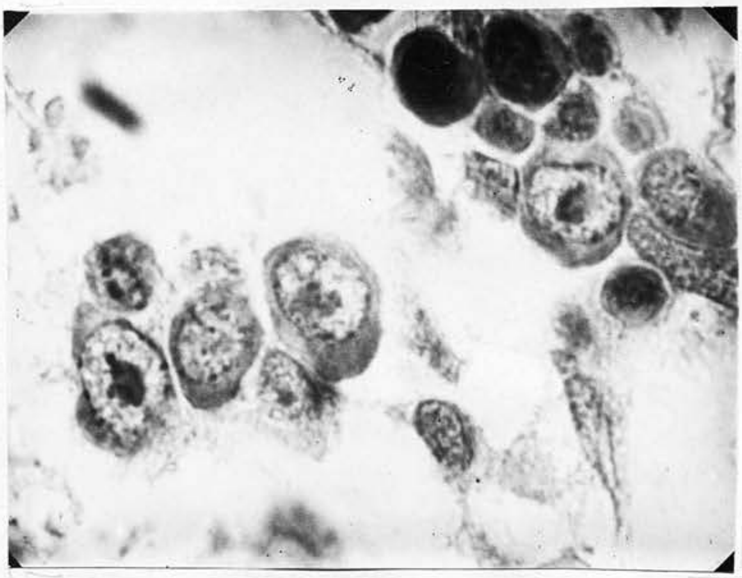

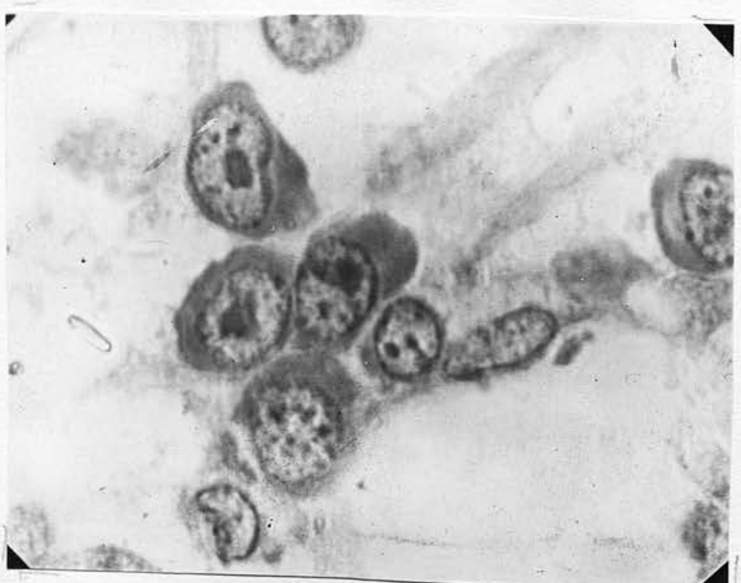

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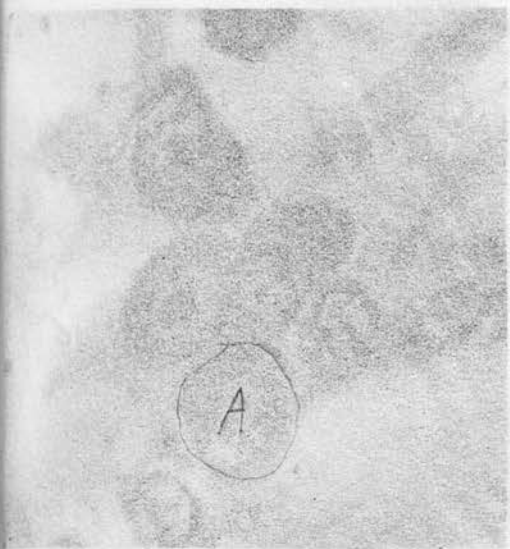

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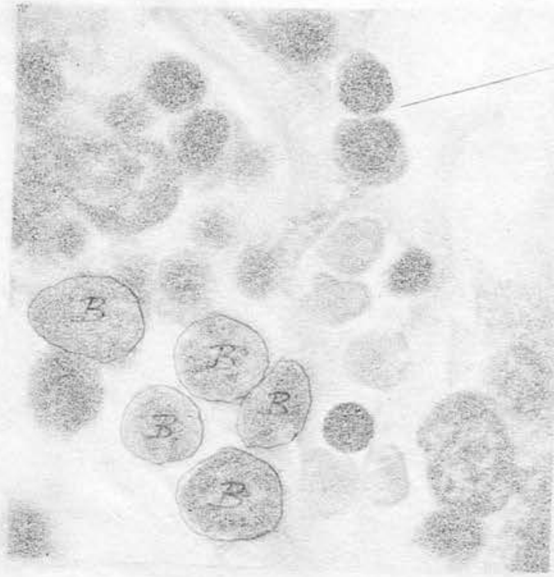

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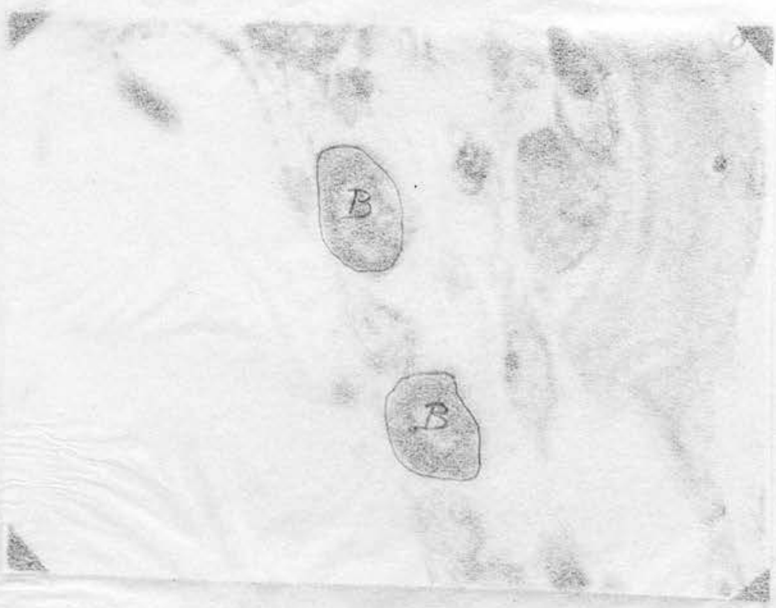
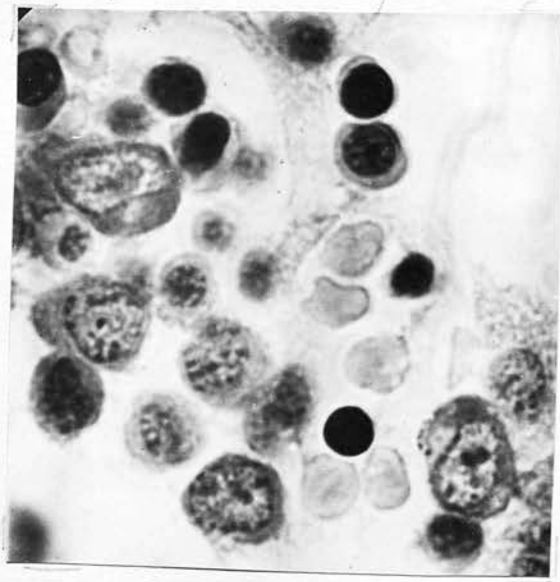


Fig XVIIb

Fig XVIII



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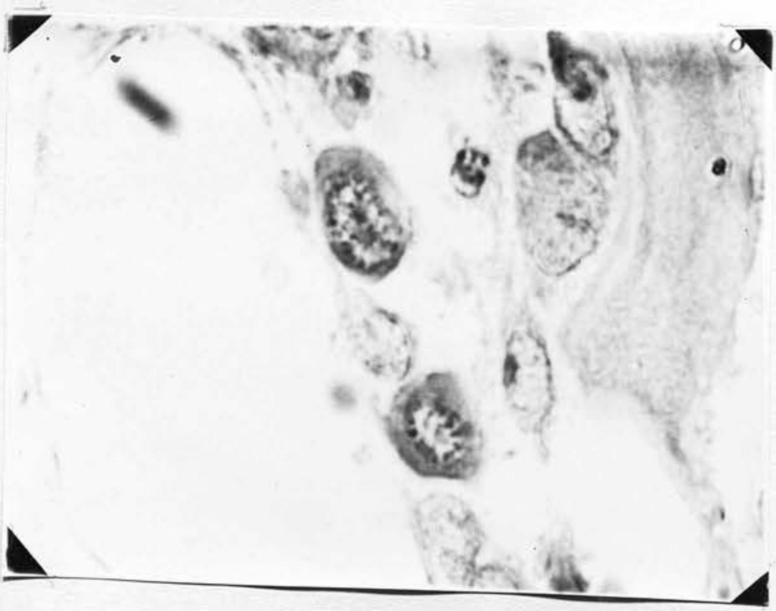

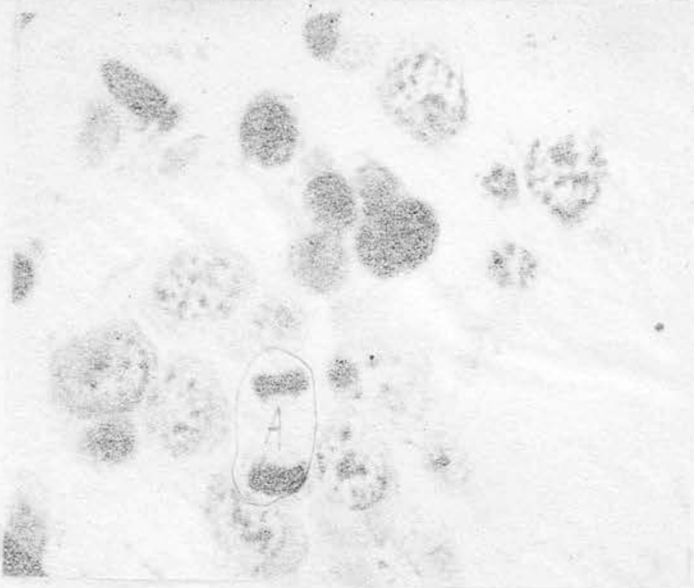
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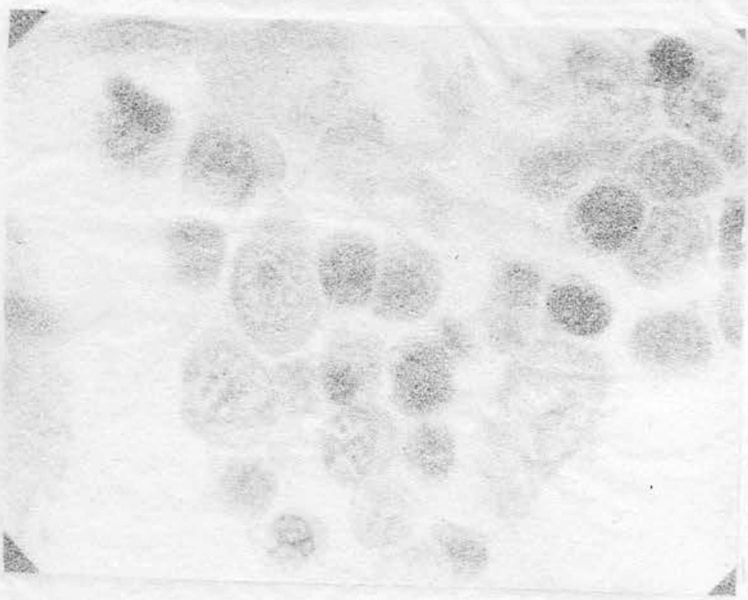

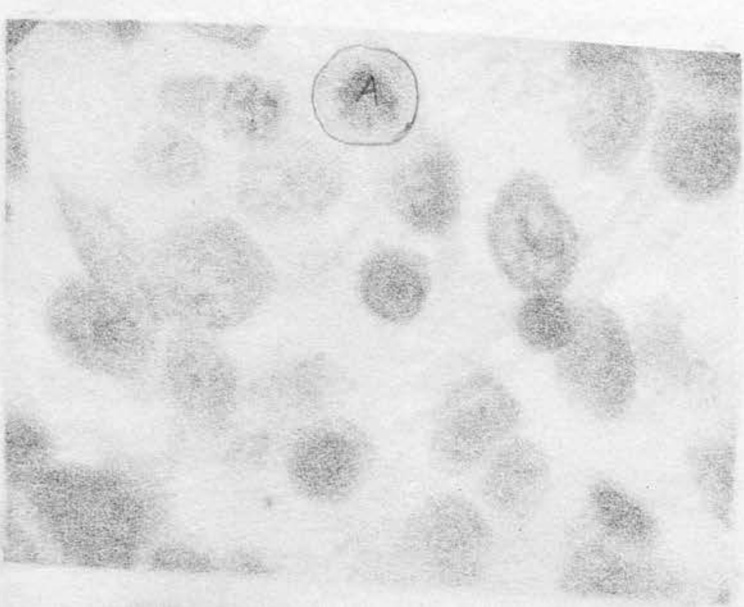
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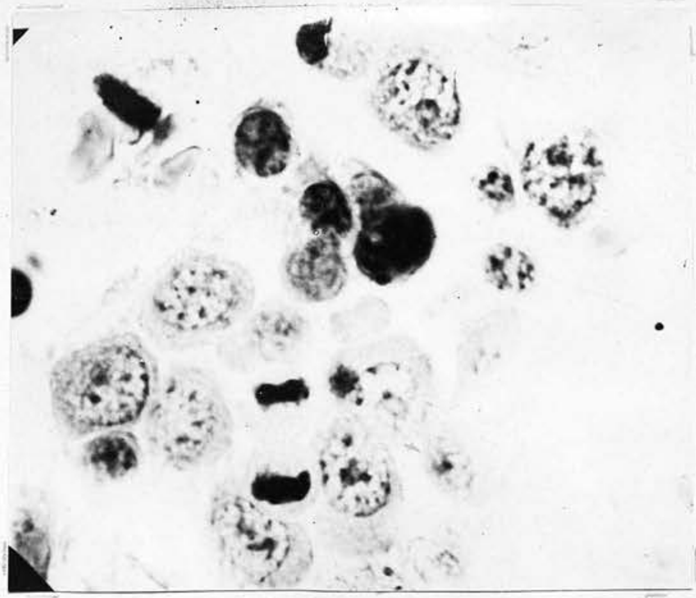
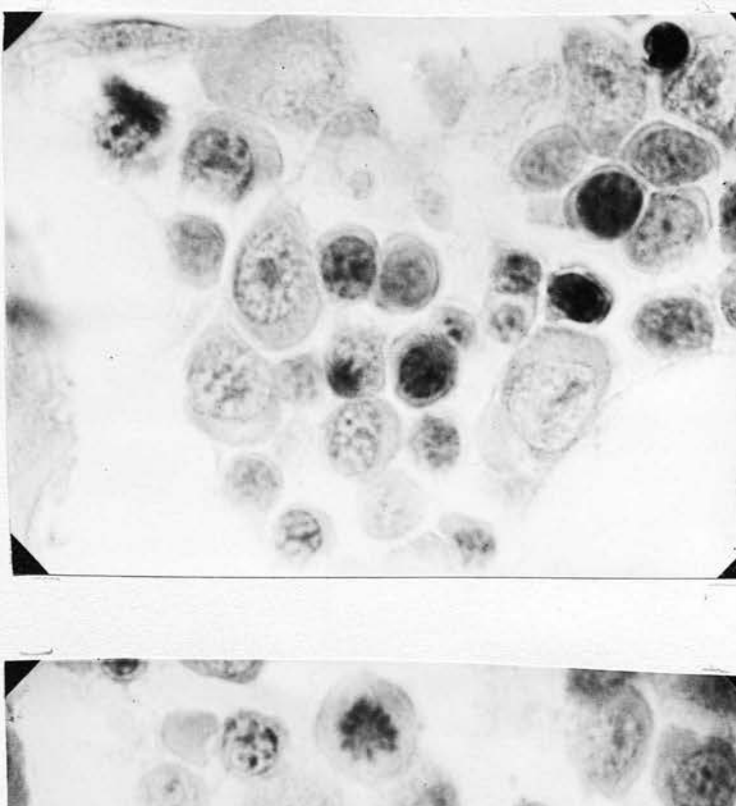
Figs XX XXI XXII





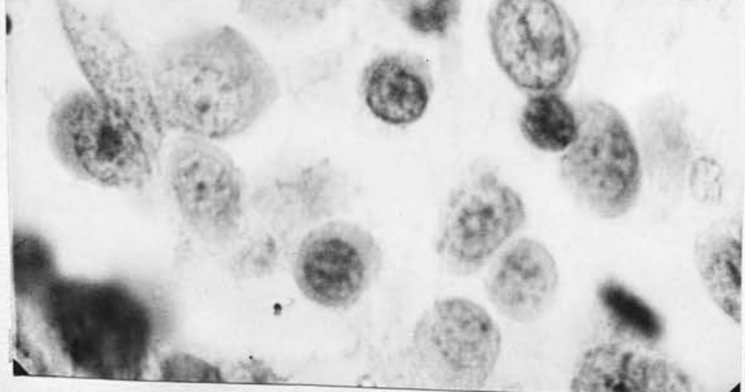
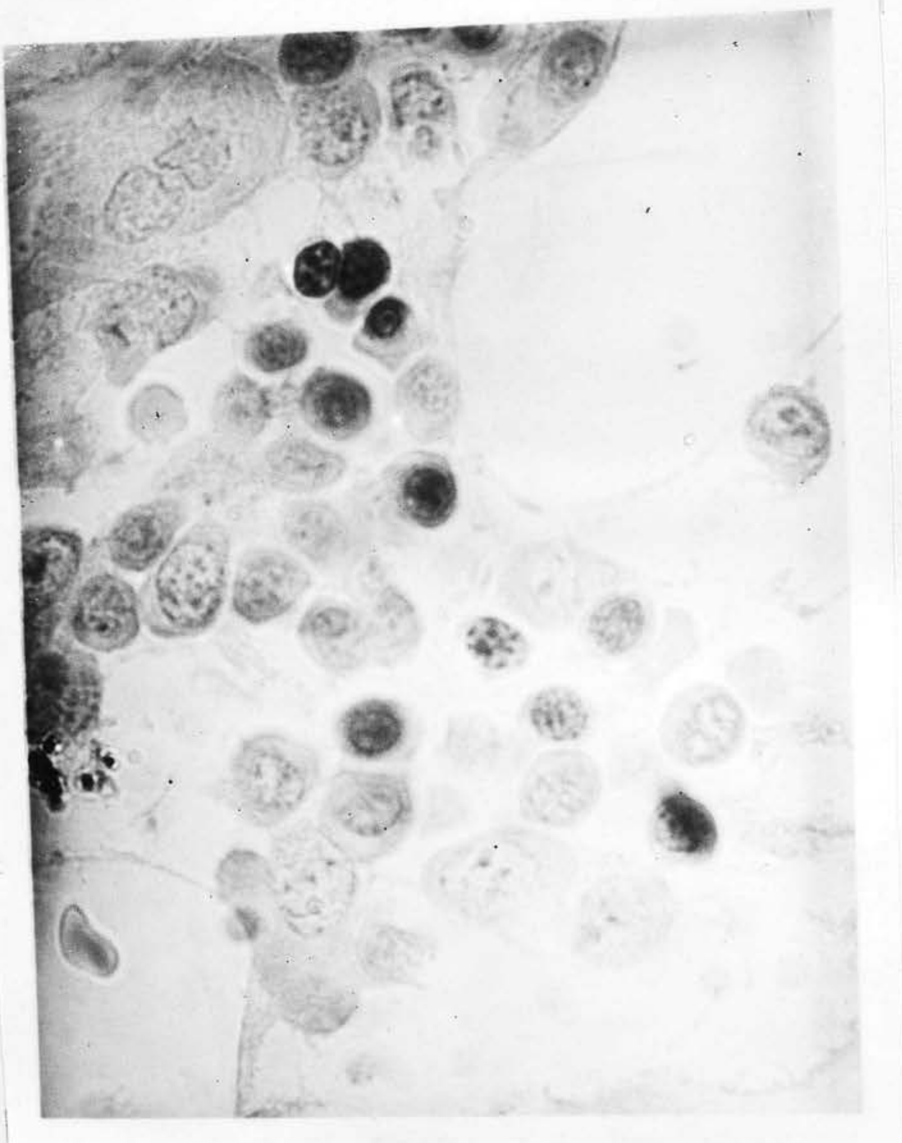


Fig. XXIII

Fig XXIII



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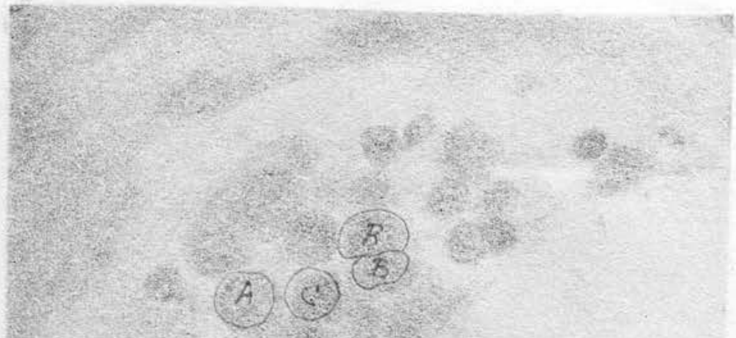
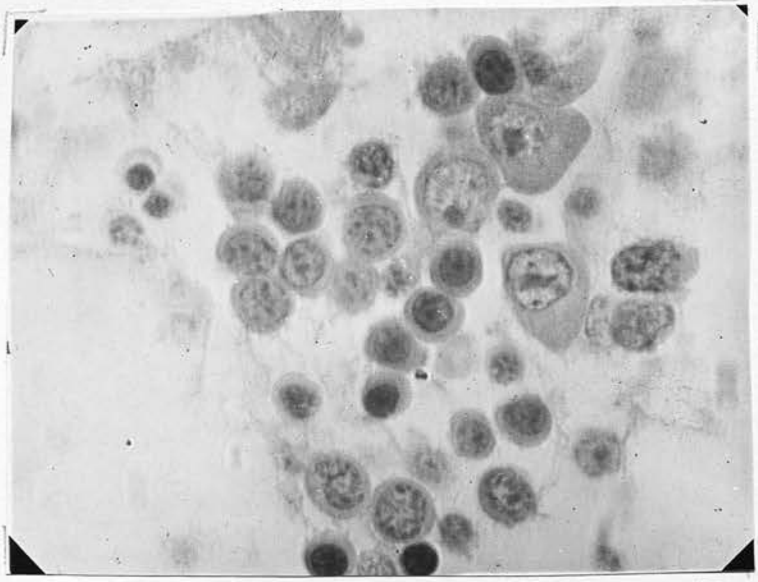


Fig XXIV



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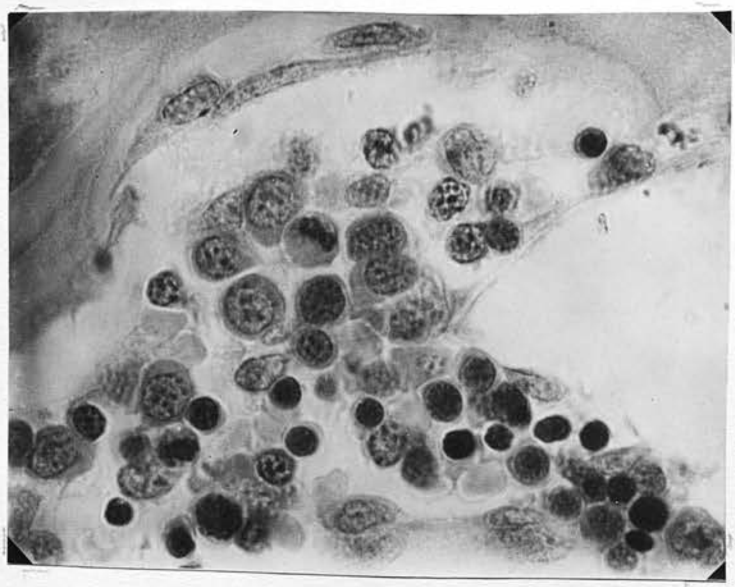

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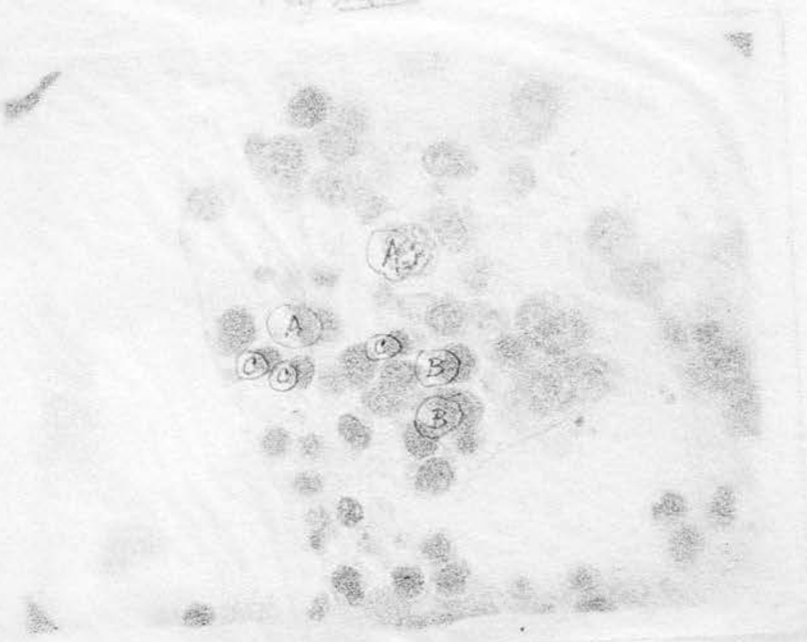
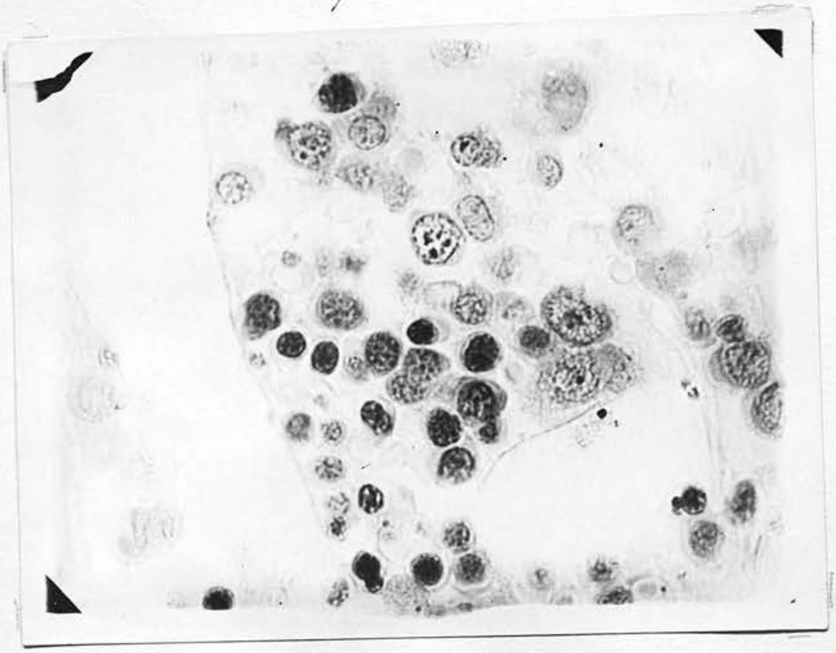


Fig XXVII

Fig XXVI



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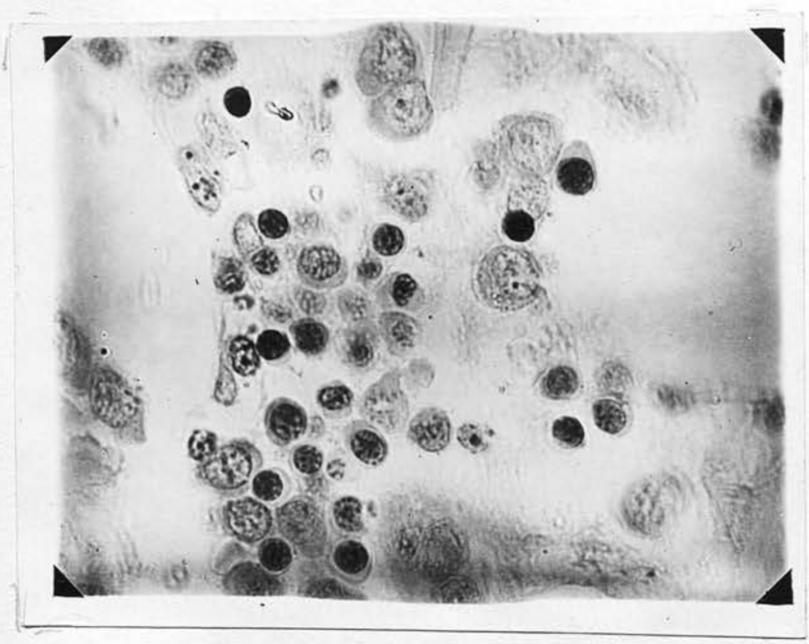

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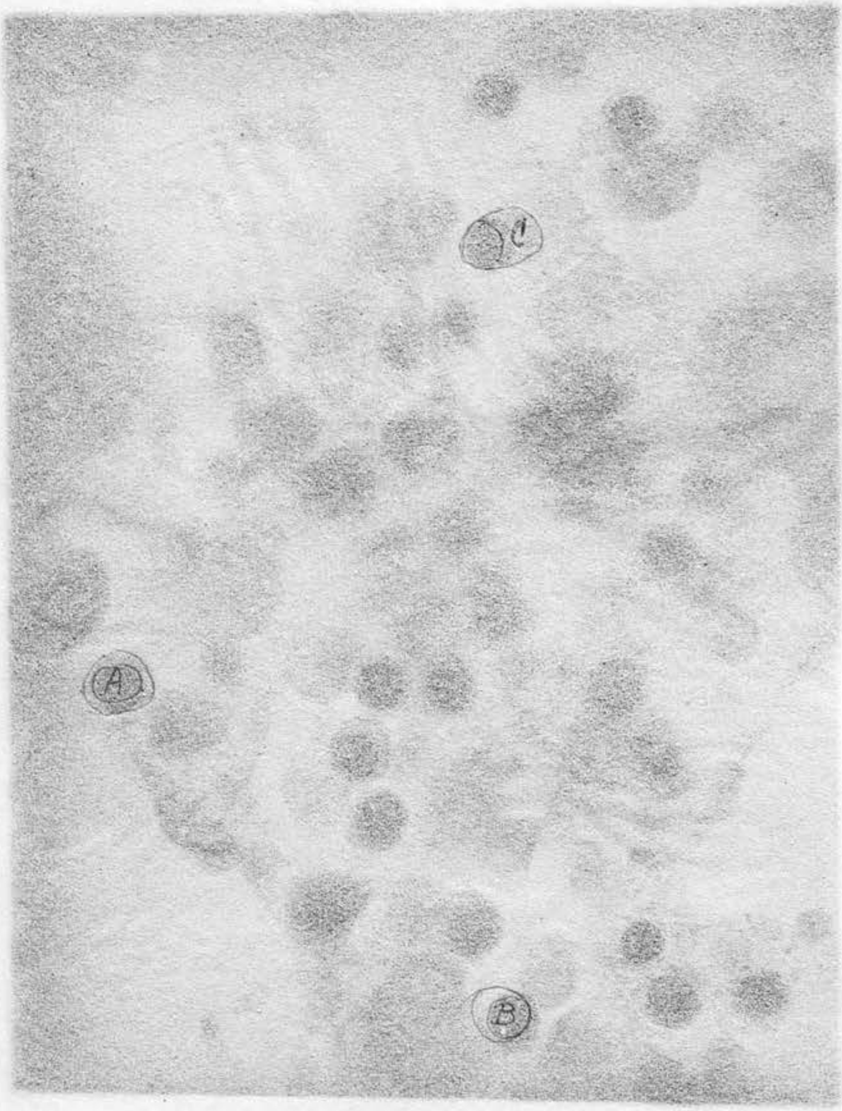

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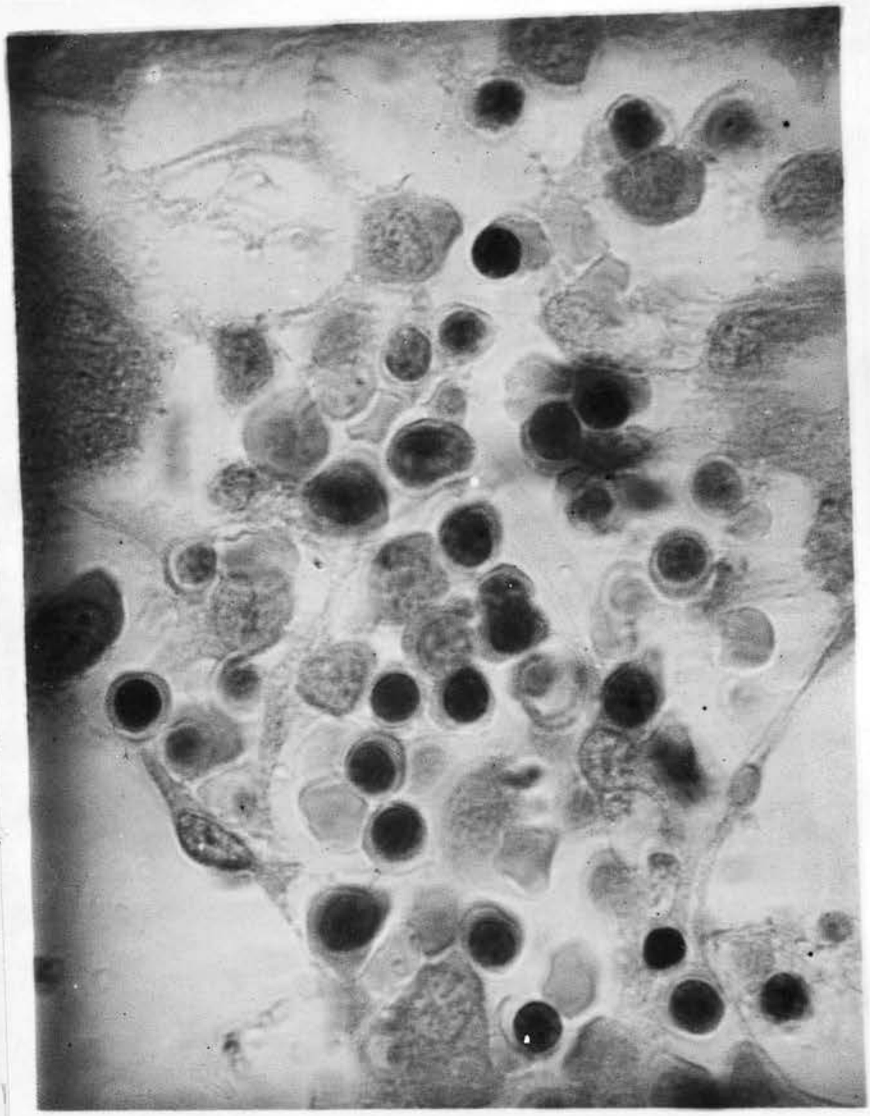

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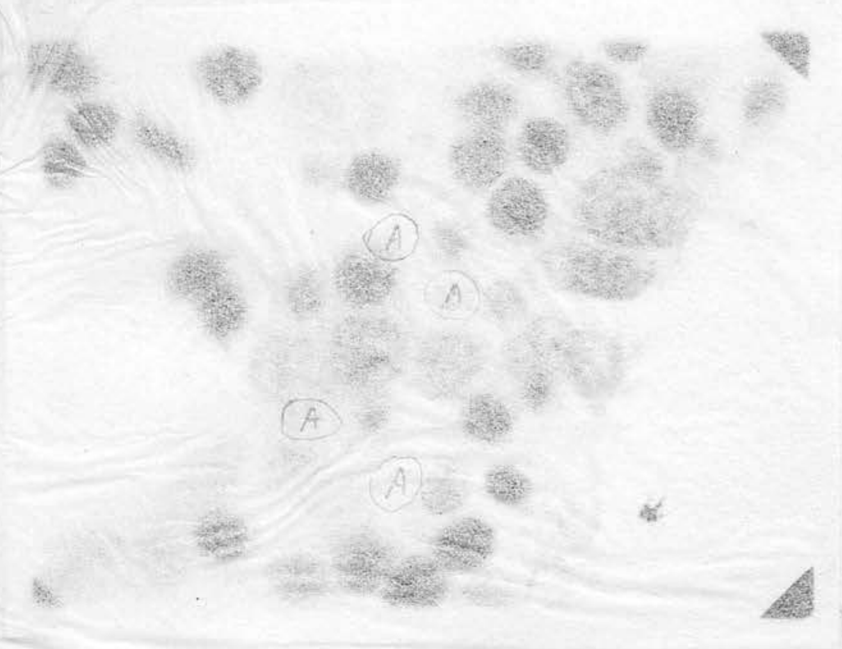

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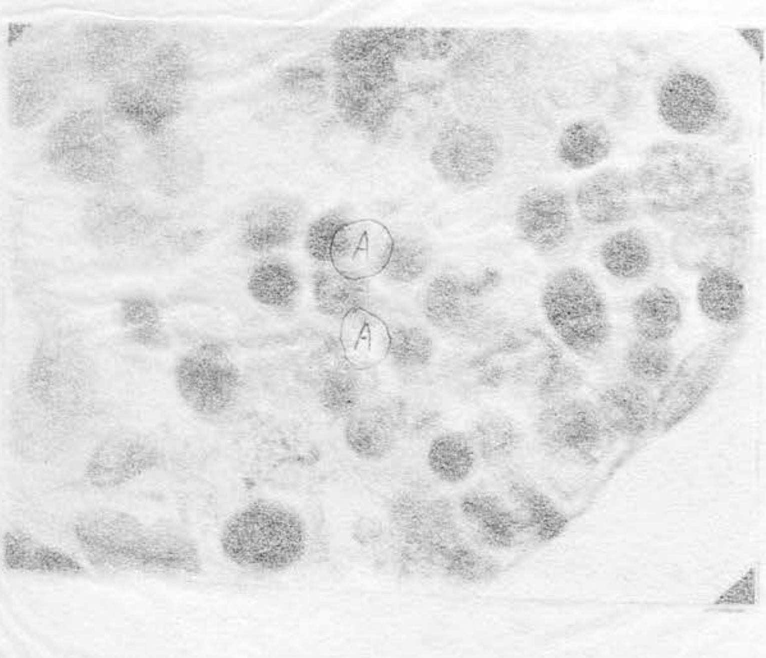

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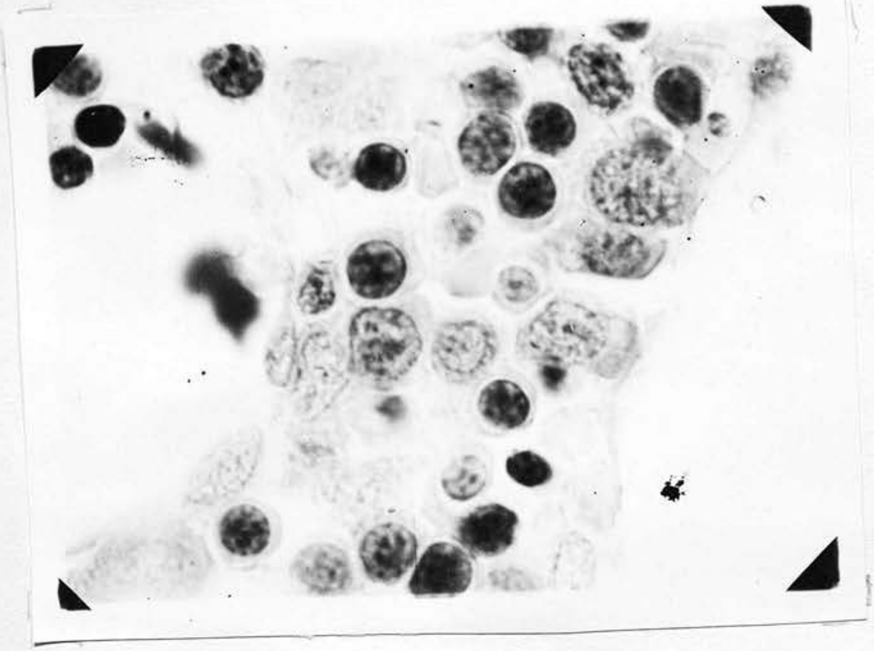

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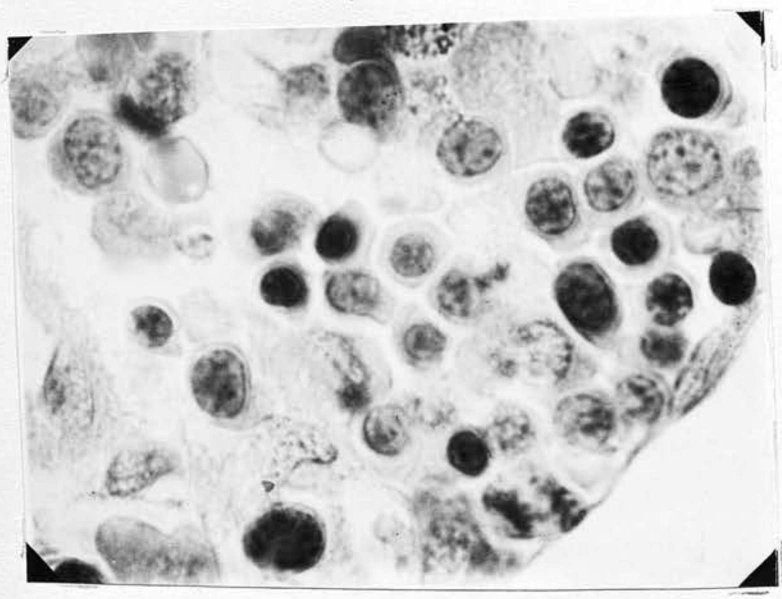

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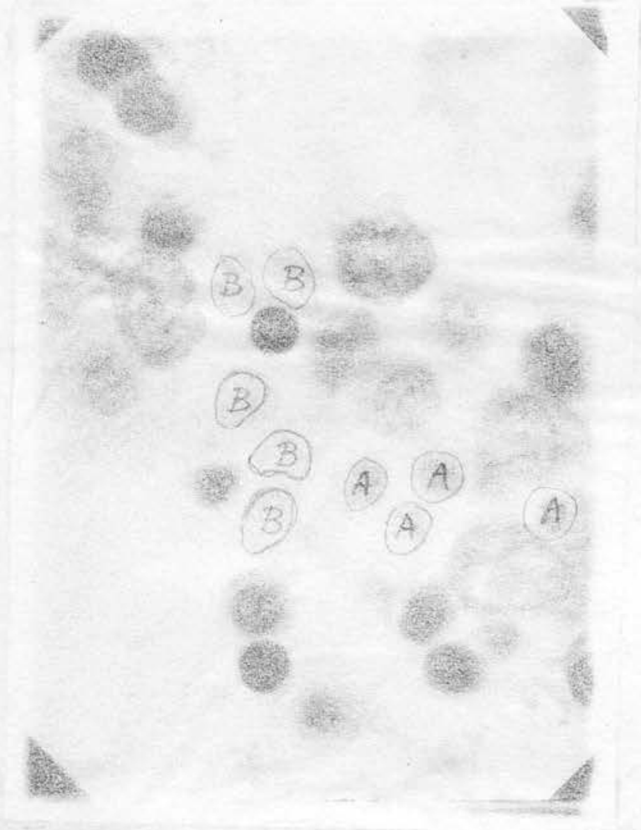

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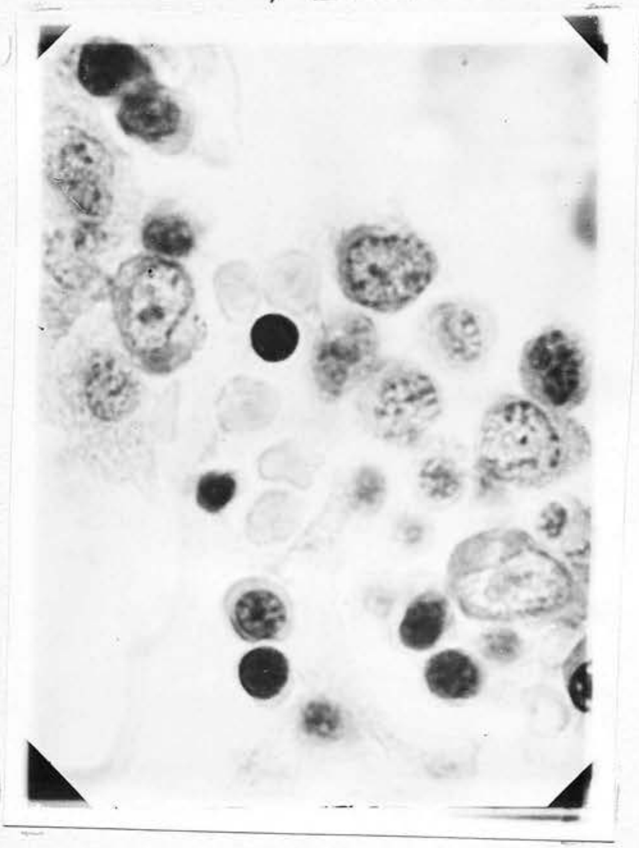

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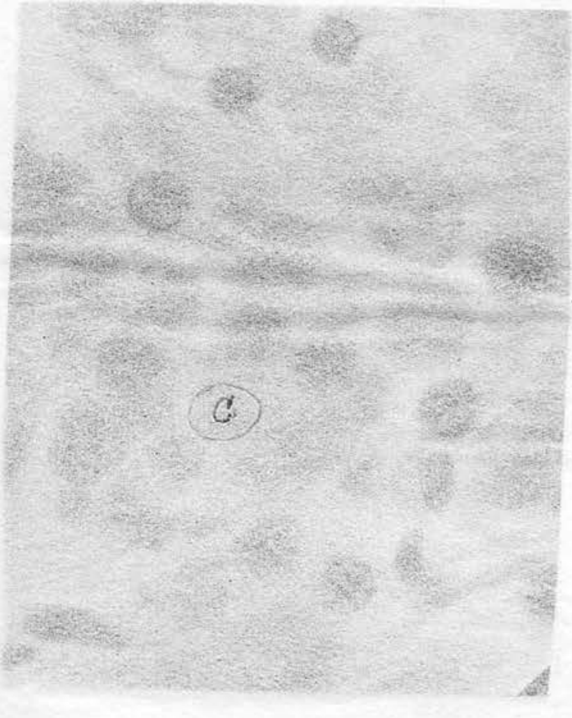

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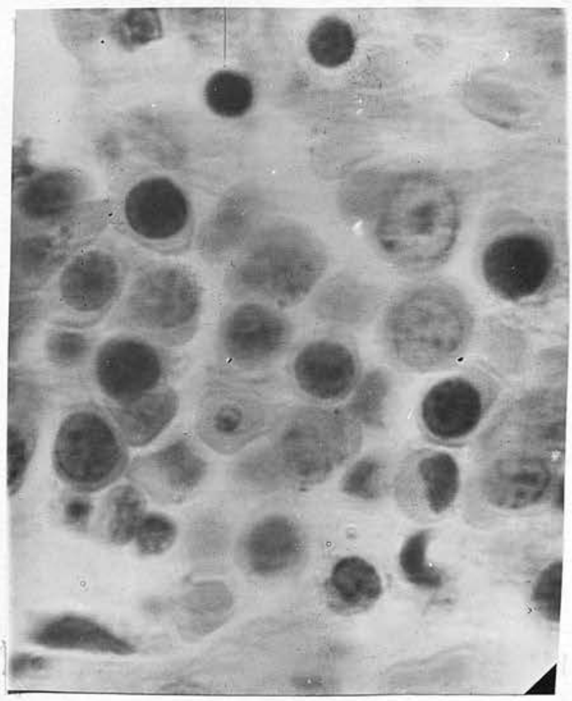

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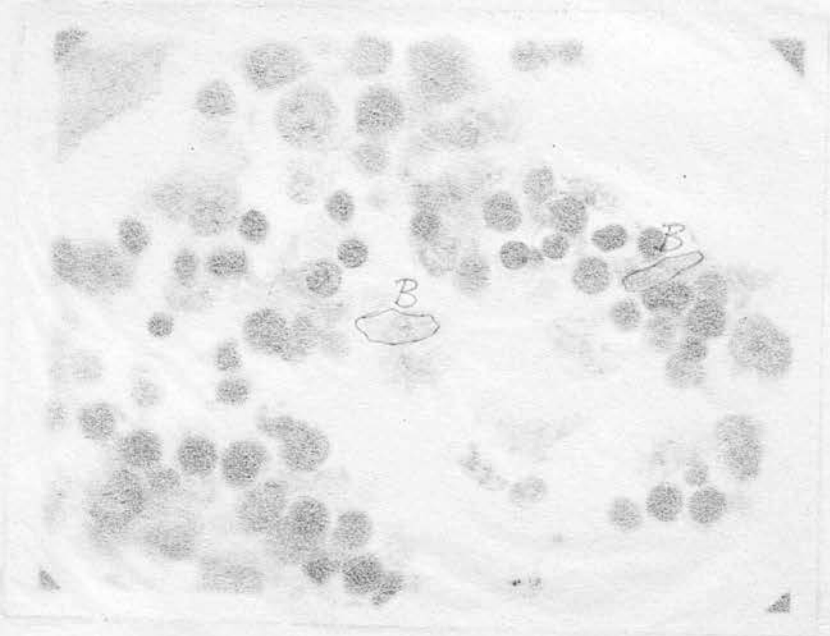

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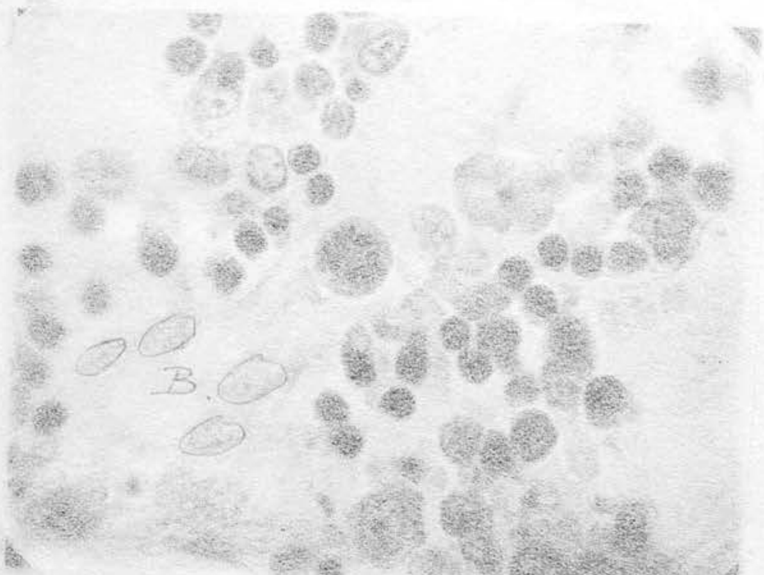

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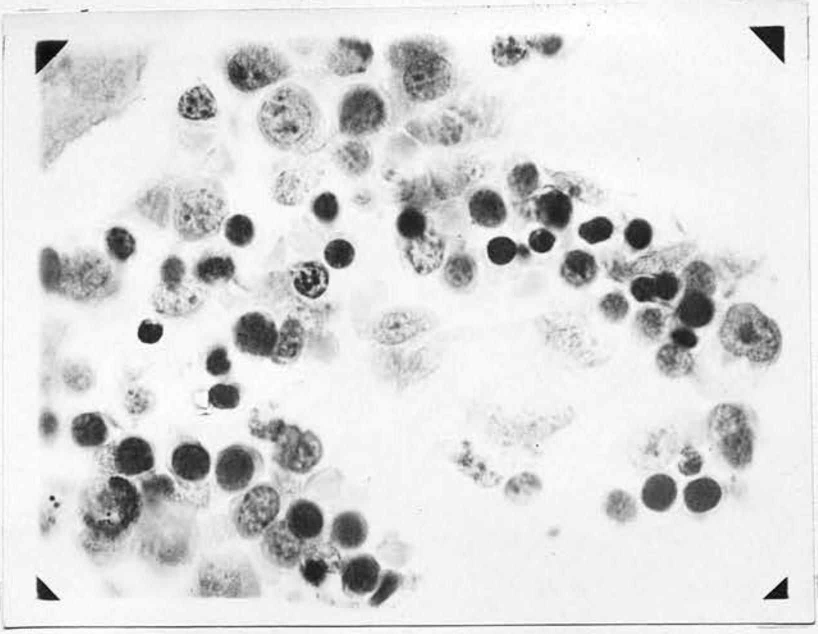

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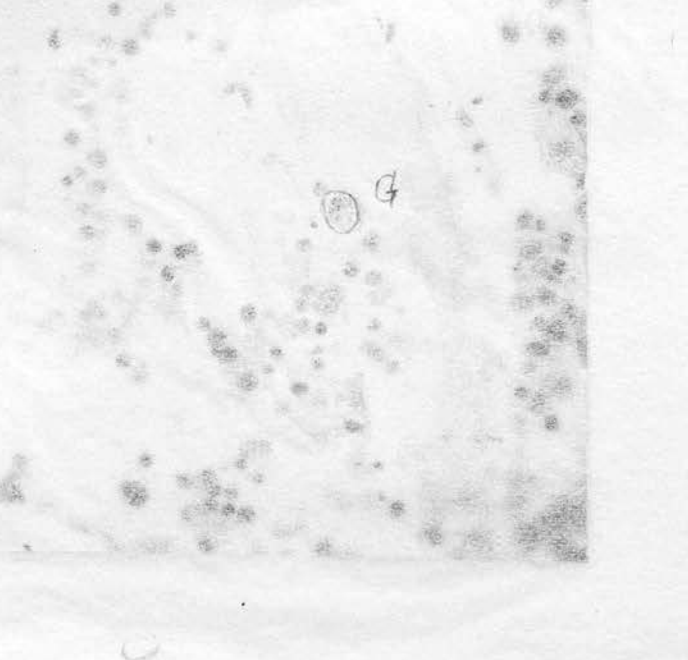

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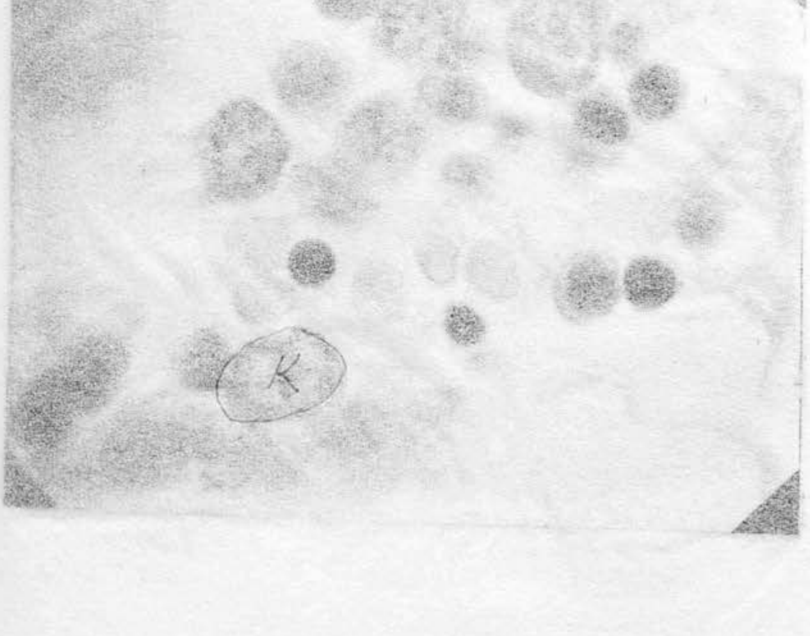

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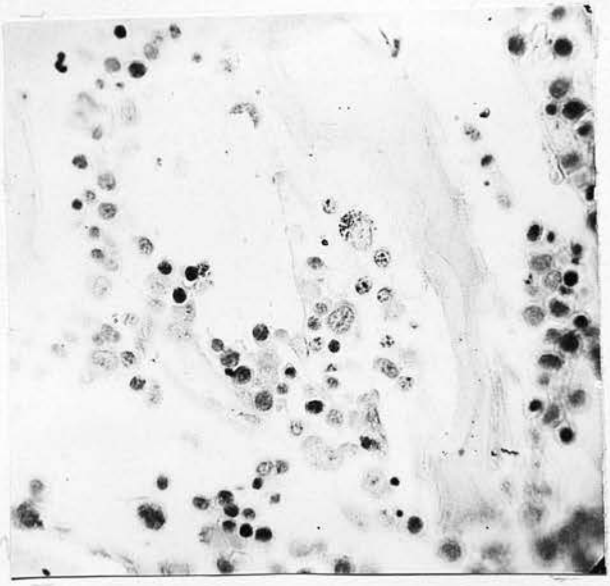

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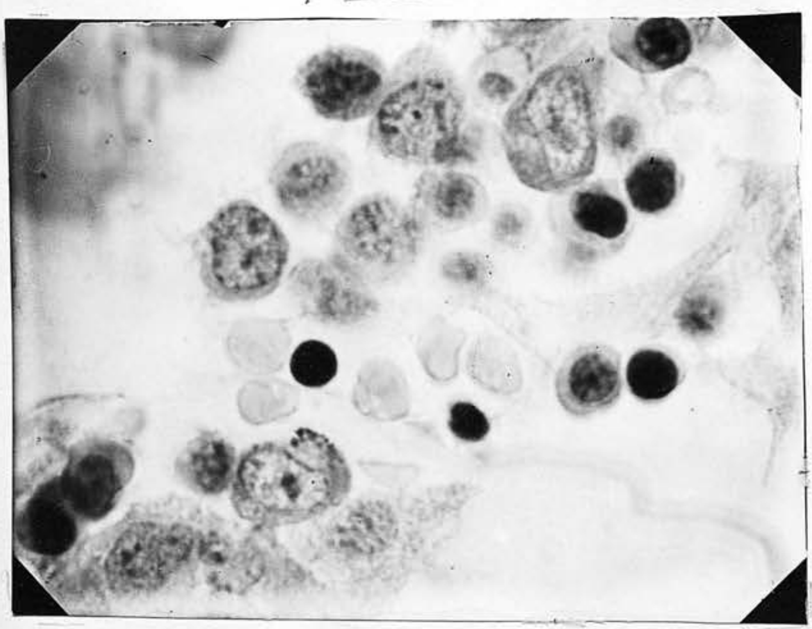

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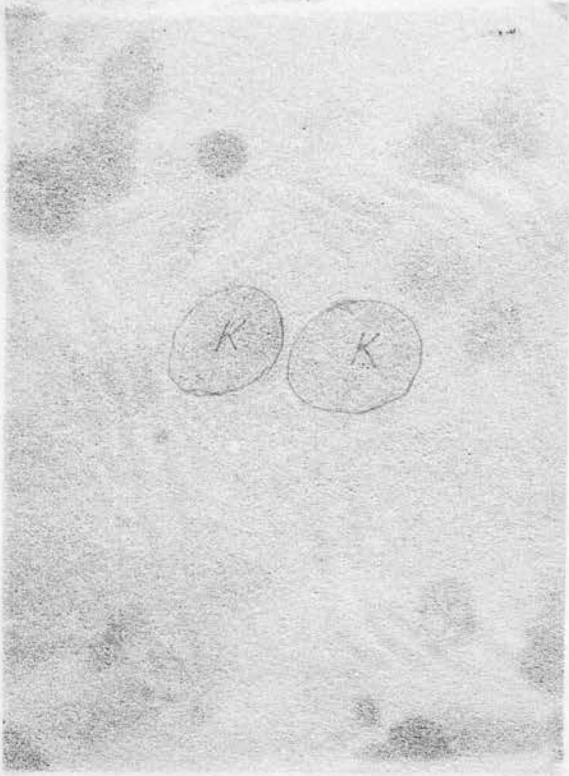

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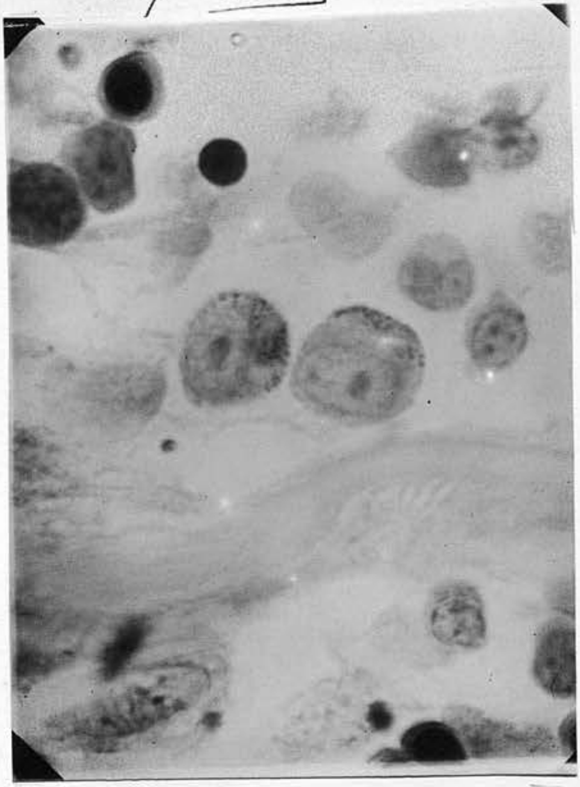
Fig XXXVII



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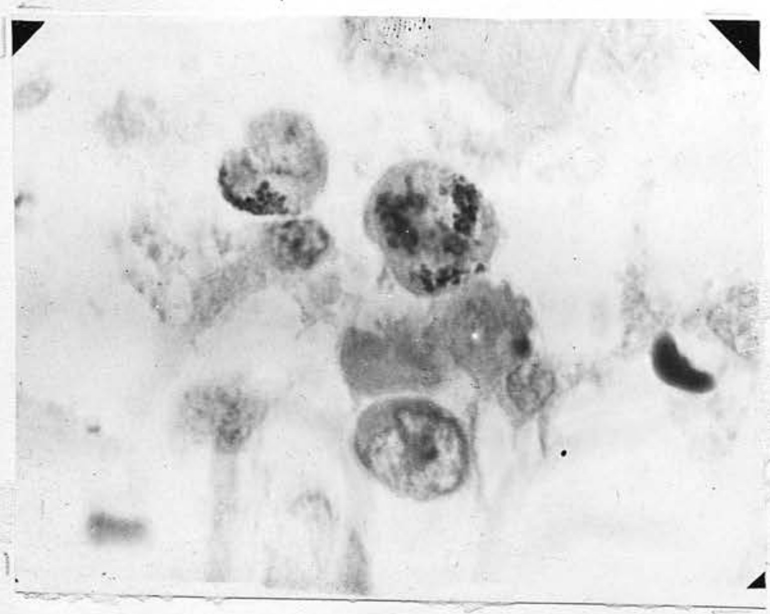

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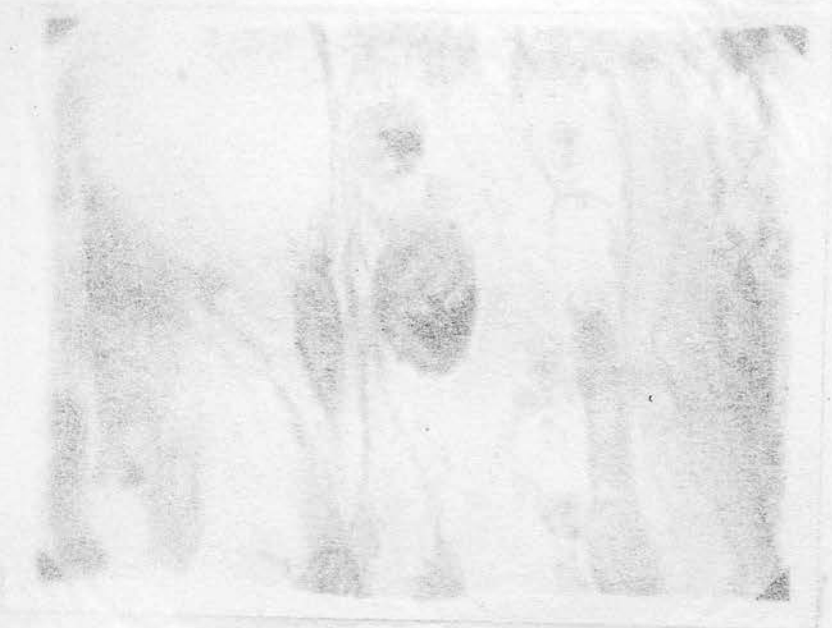
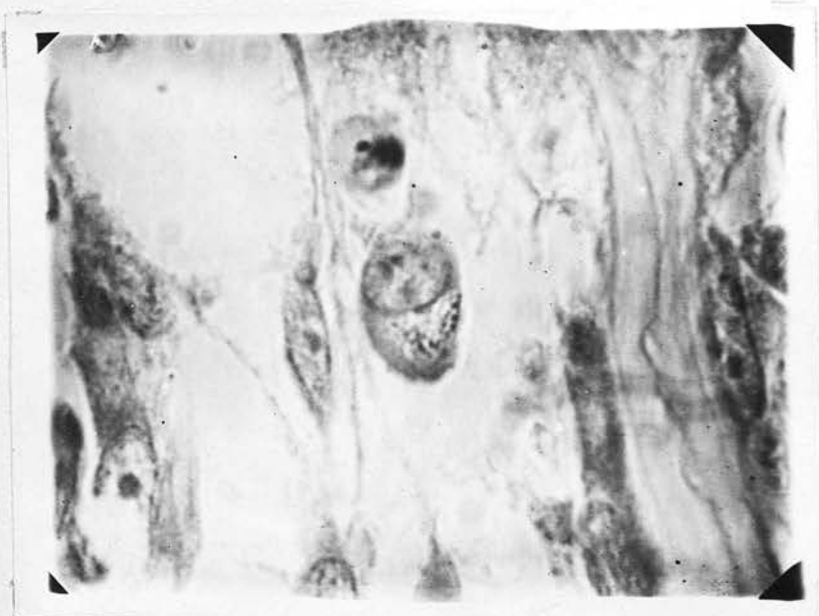
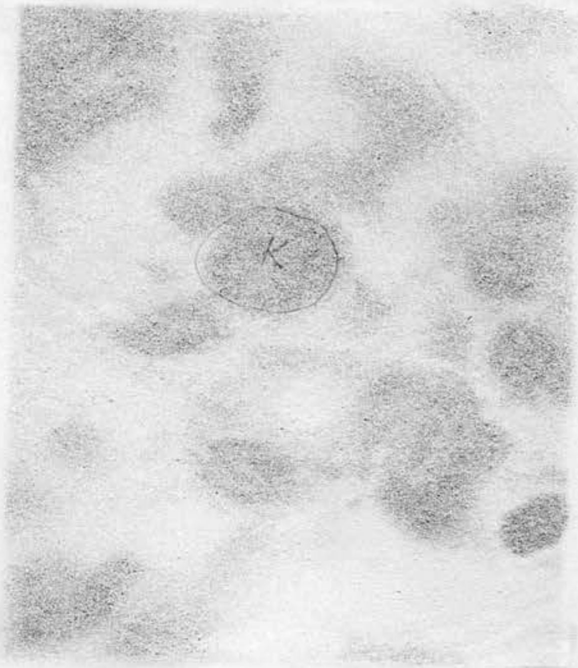


Fig XL

Fig XXXIX

Fig XL

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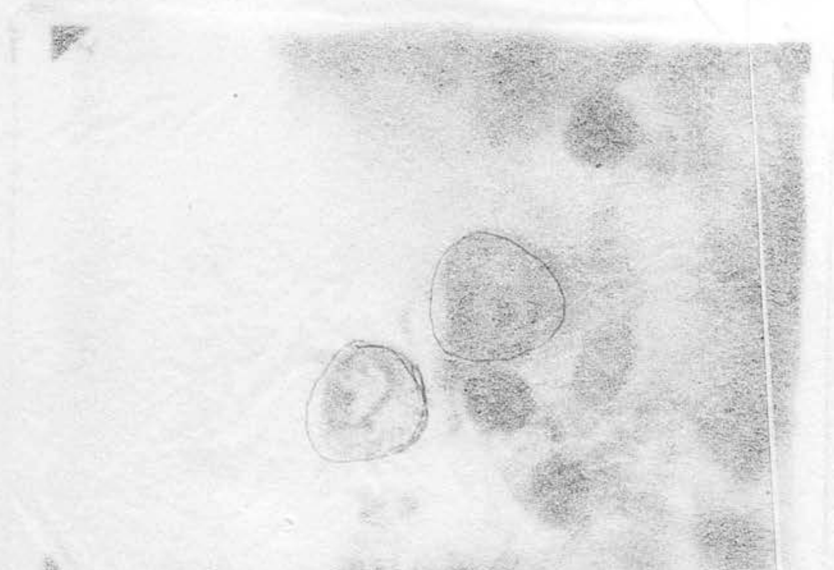

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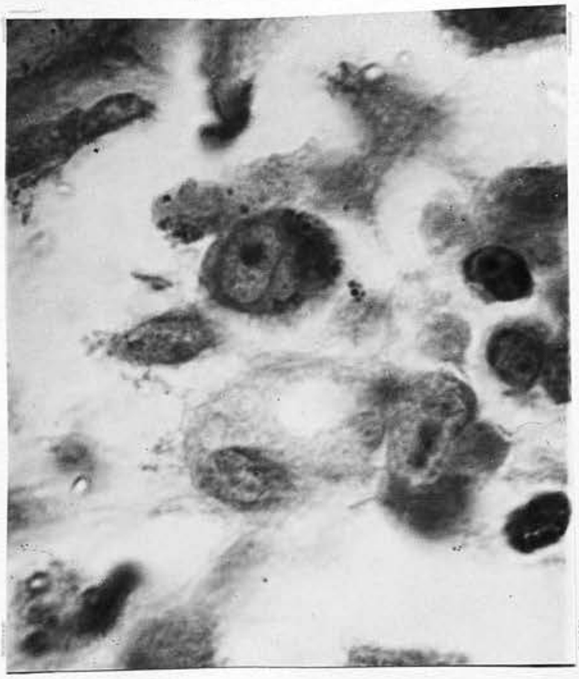

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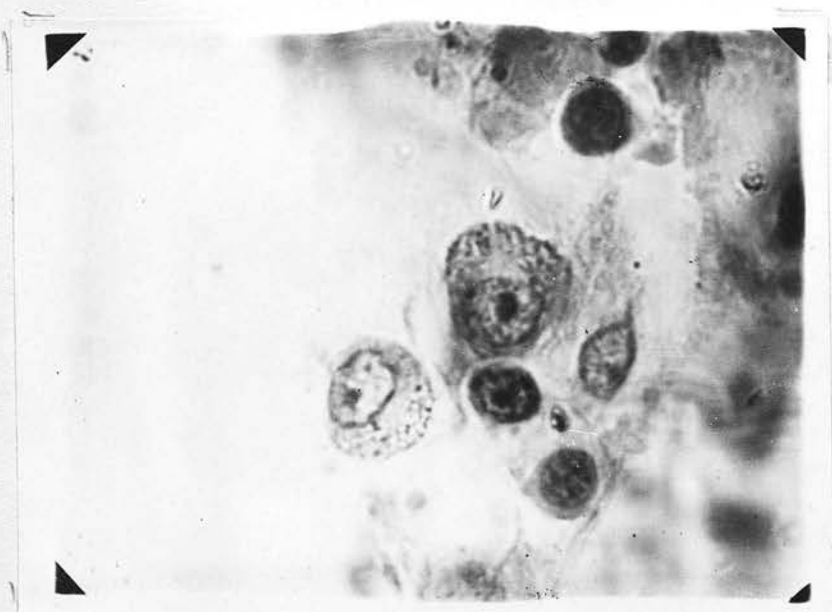

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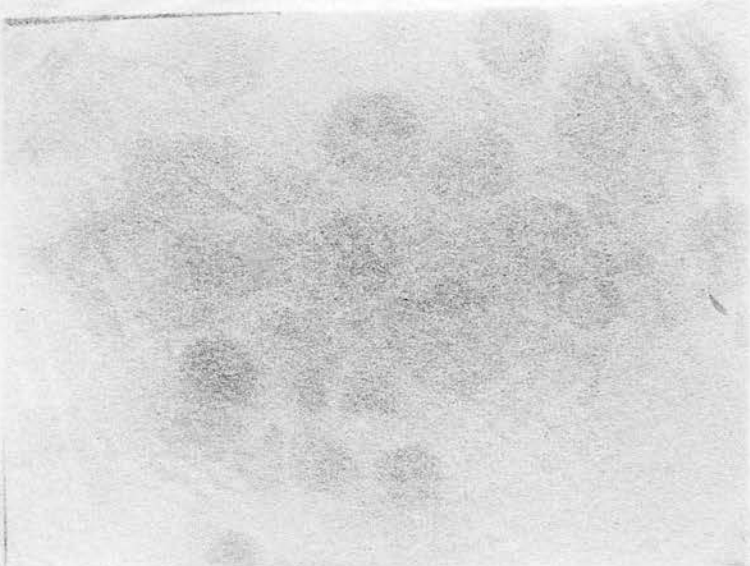

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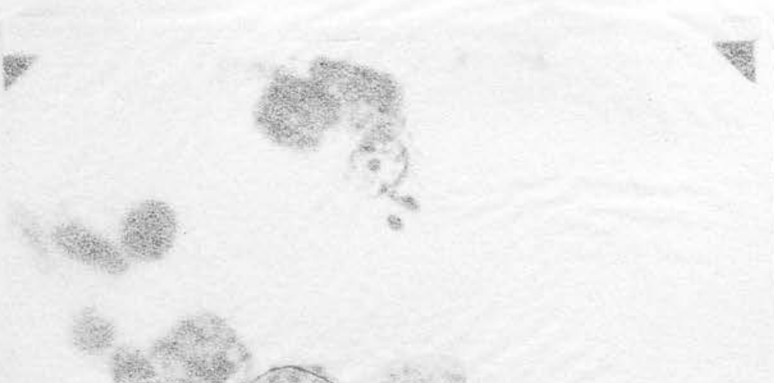

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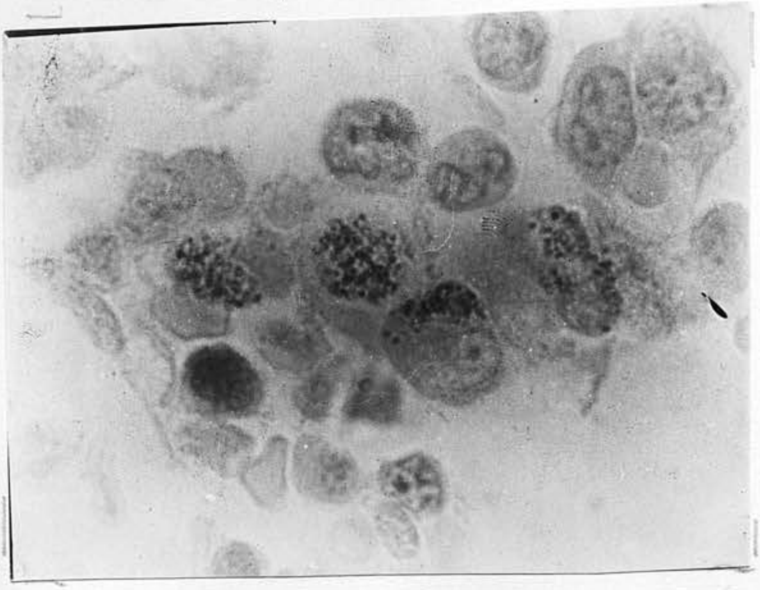

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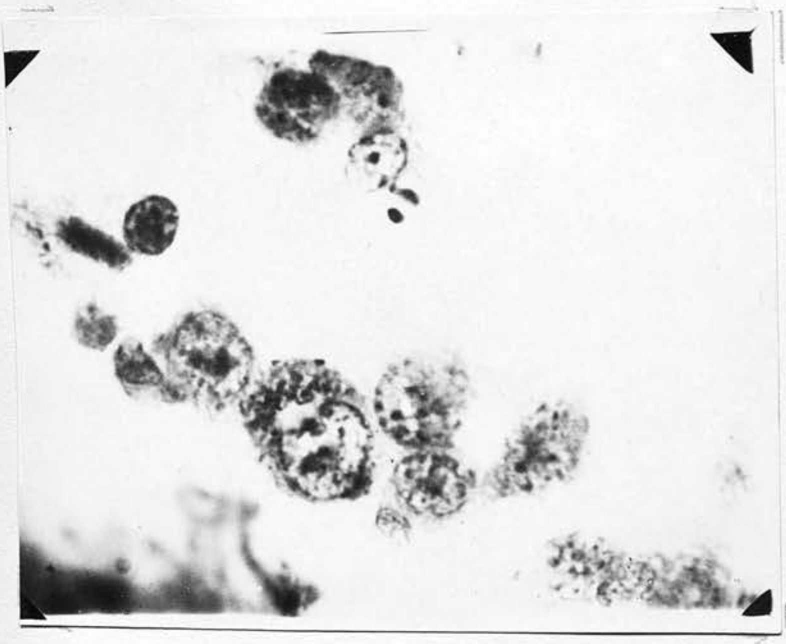


Fig XLV

Fig XLVI

Fig XLV

Fig XLVI

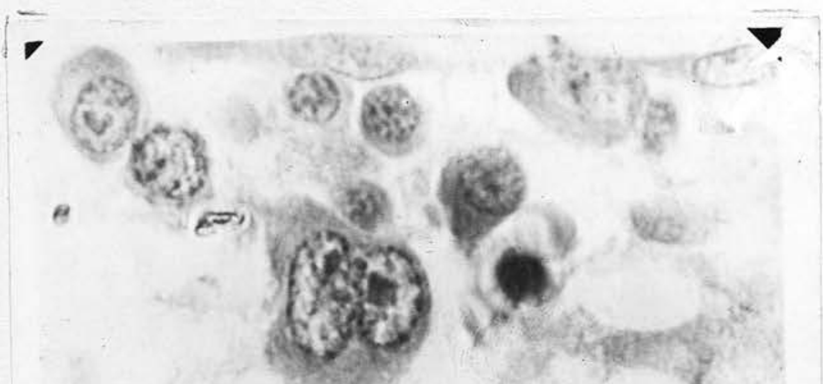


Fig. XLVII

Fig. XLVIII

Fig XLVII

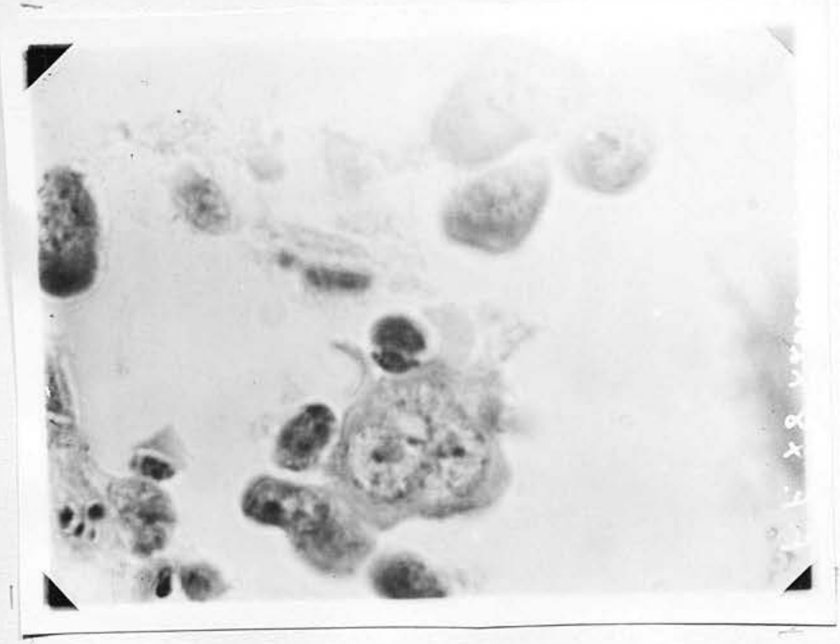


Fig XLVIII

Fig XLIX

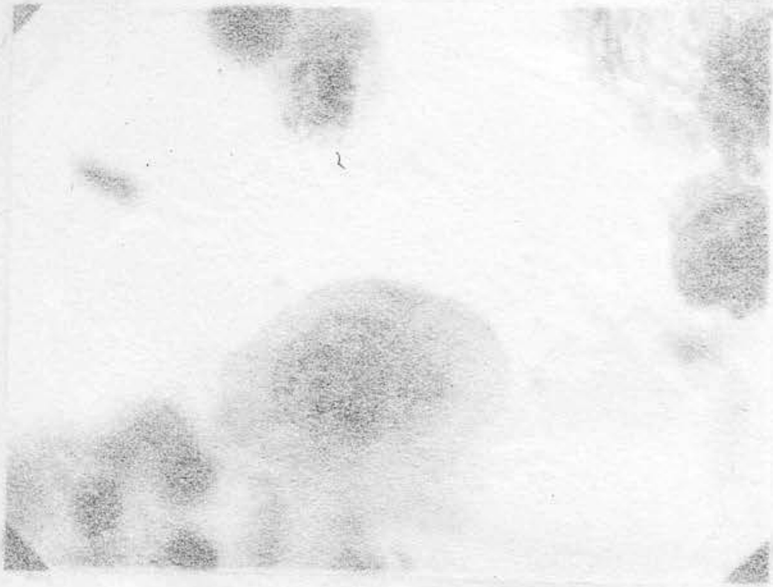
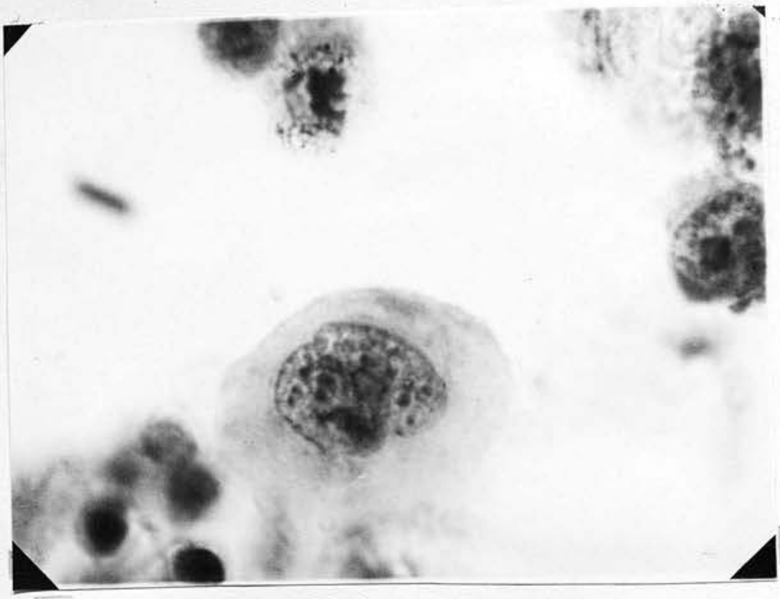


Fig L

Fig XLIX



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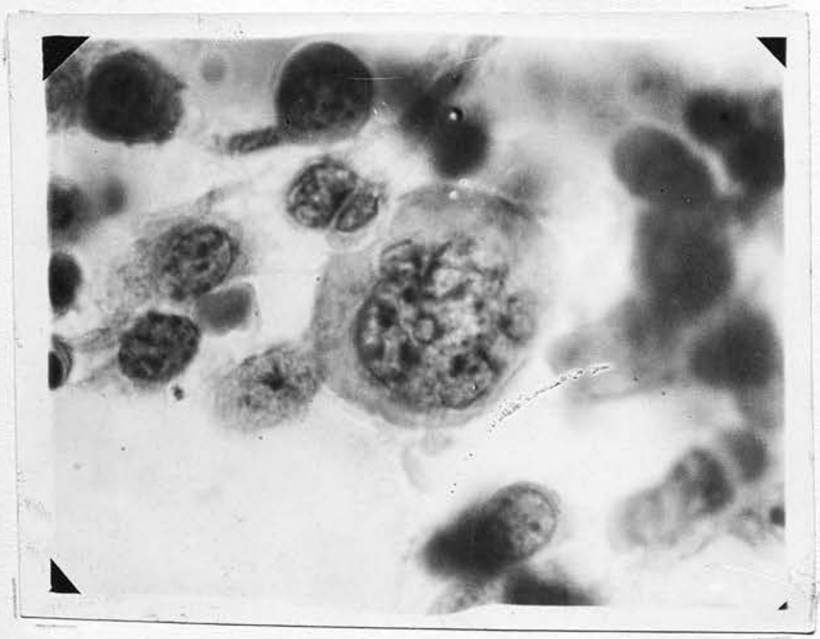
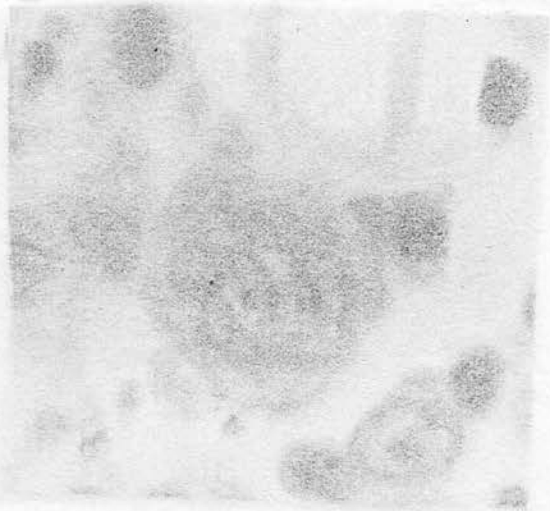
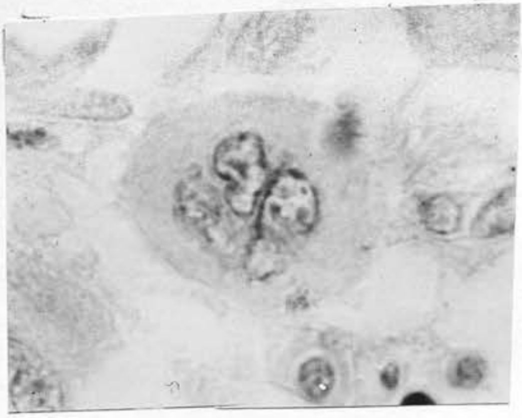
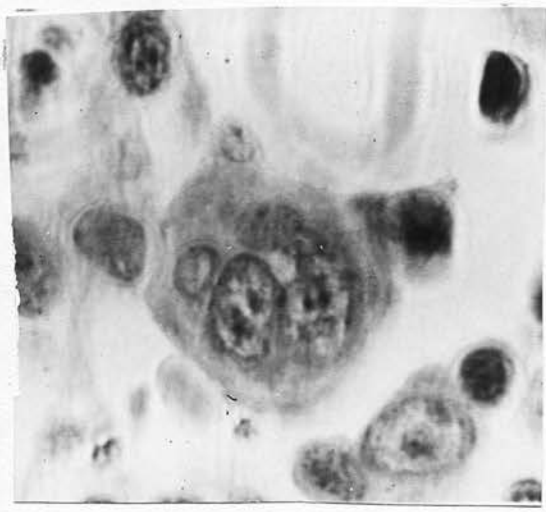


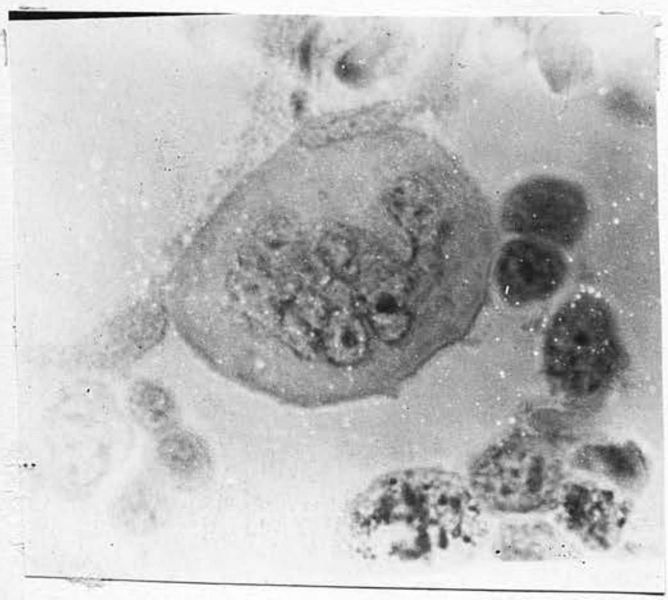
Figs LI LII LIII



Figs LI LII. LIII







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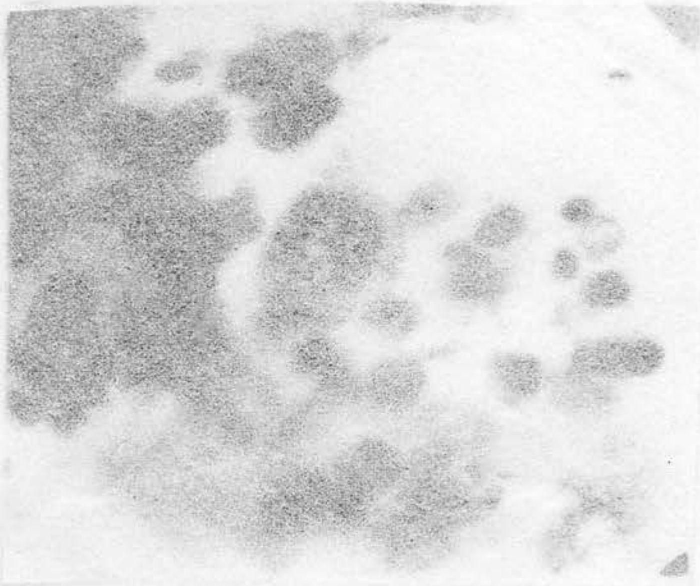
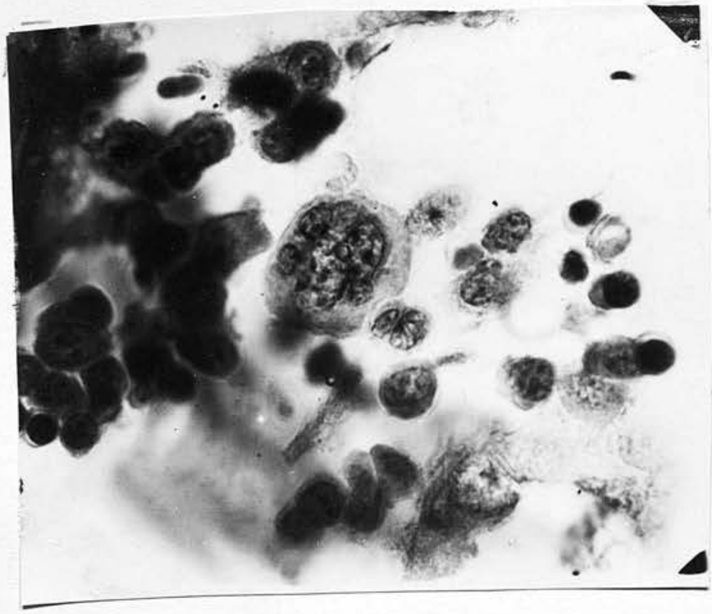


Fig XV.

Fig LIV



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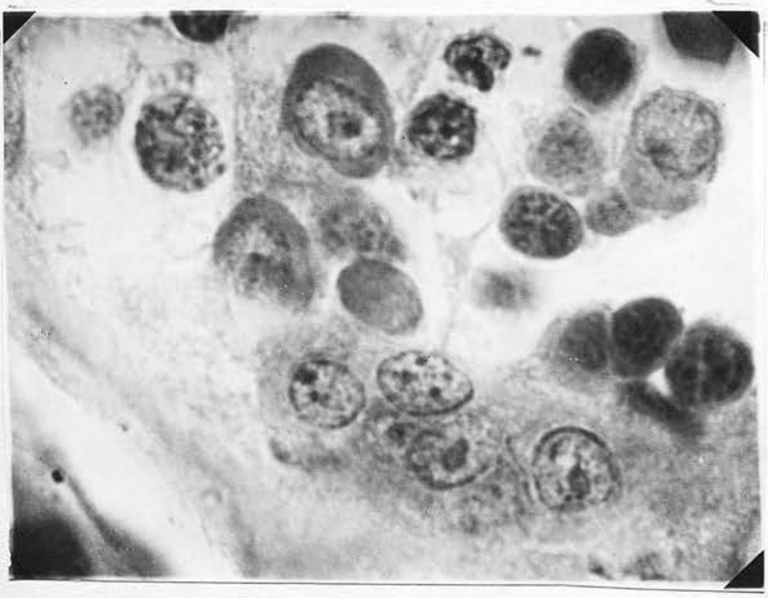

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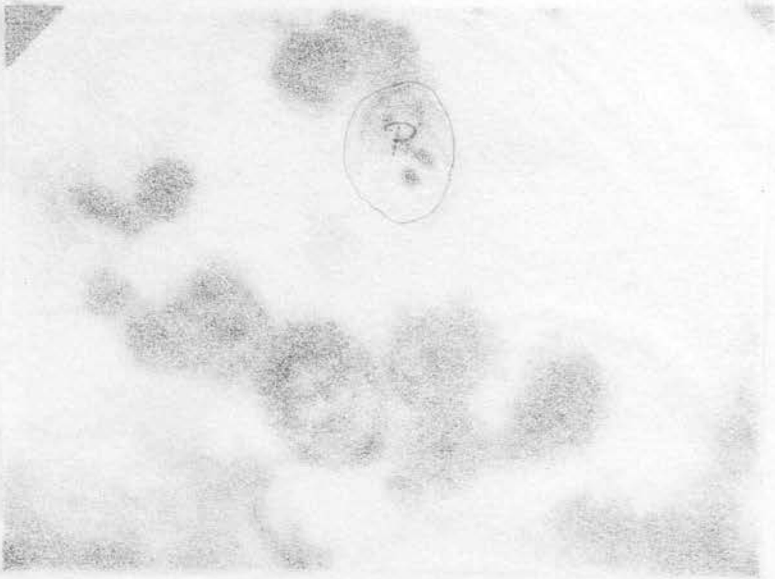
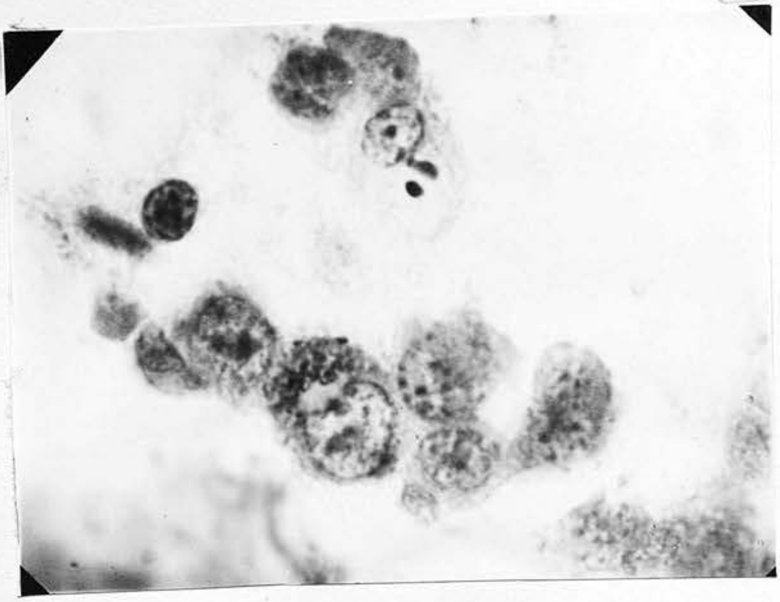


Fig XVII

Fig. XVI



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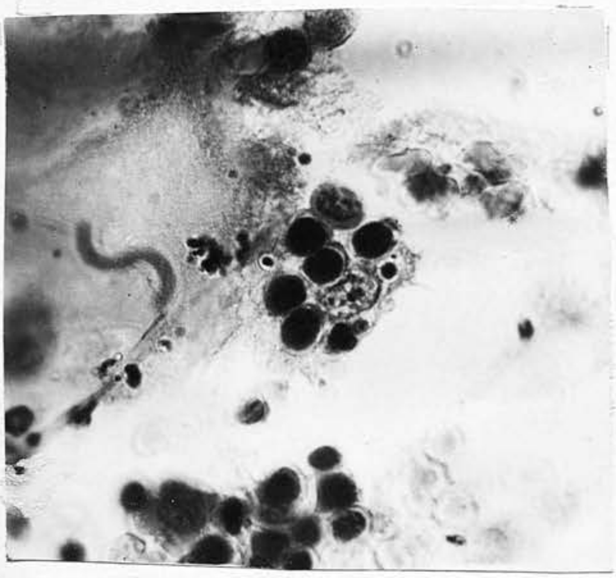

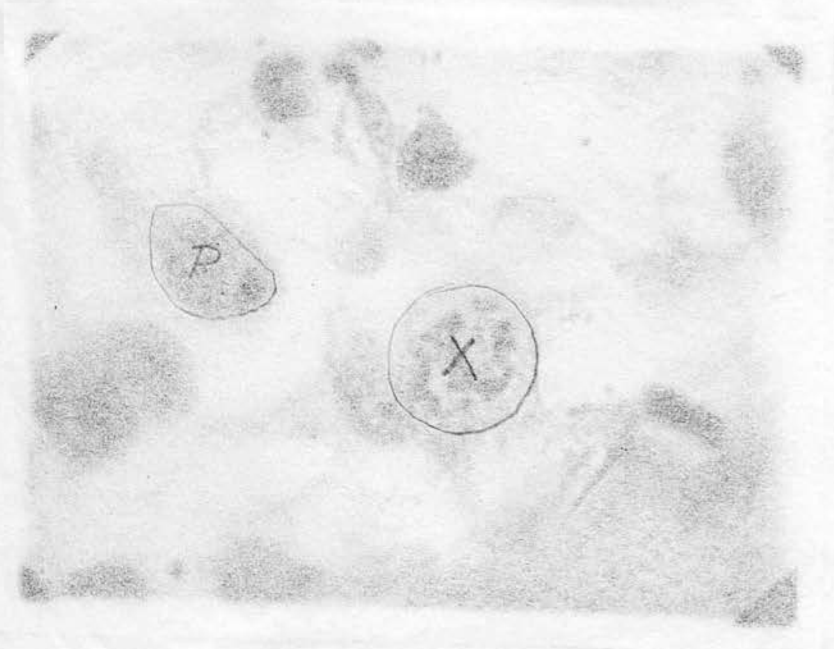
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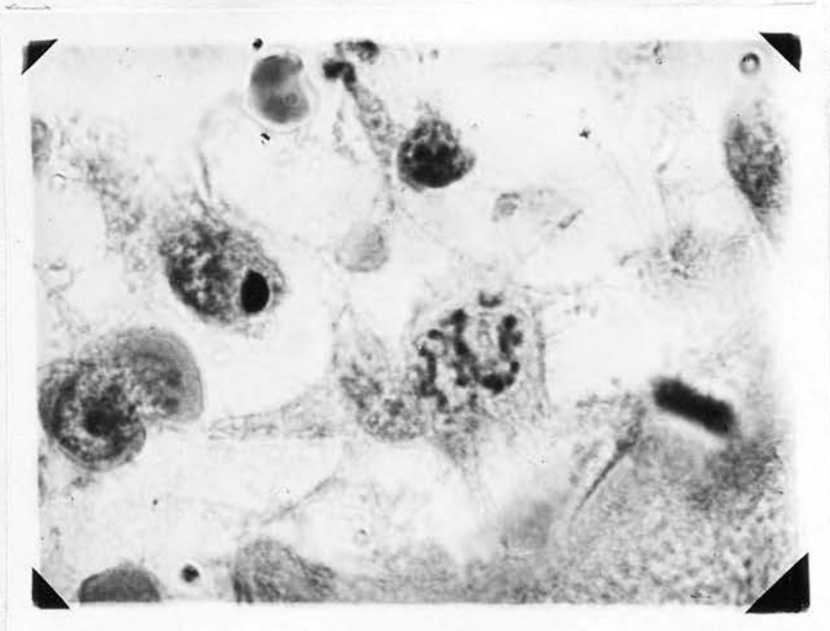

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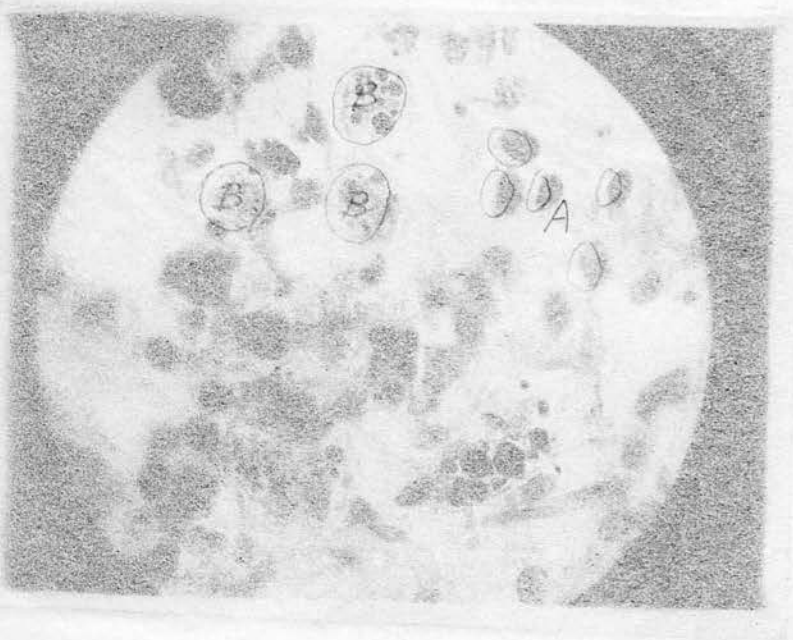


Fig L·IX

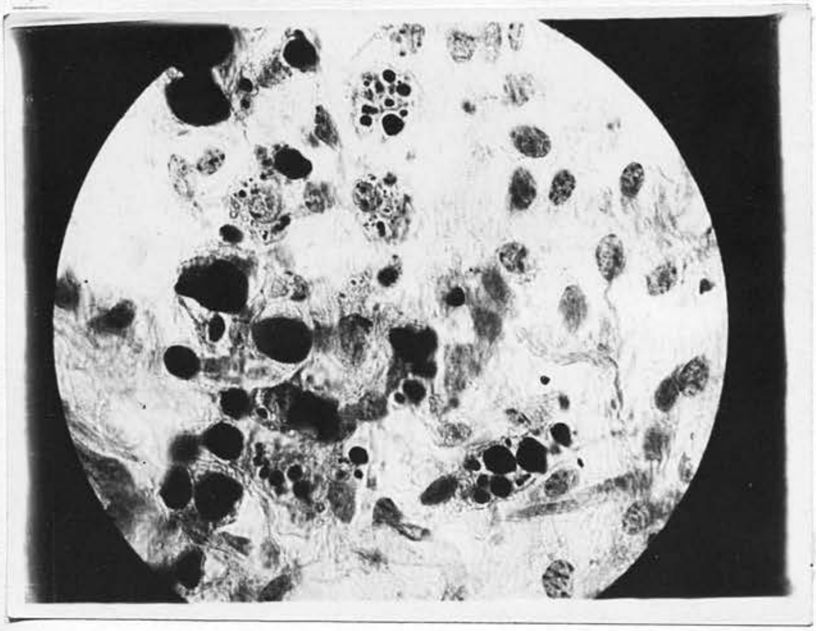
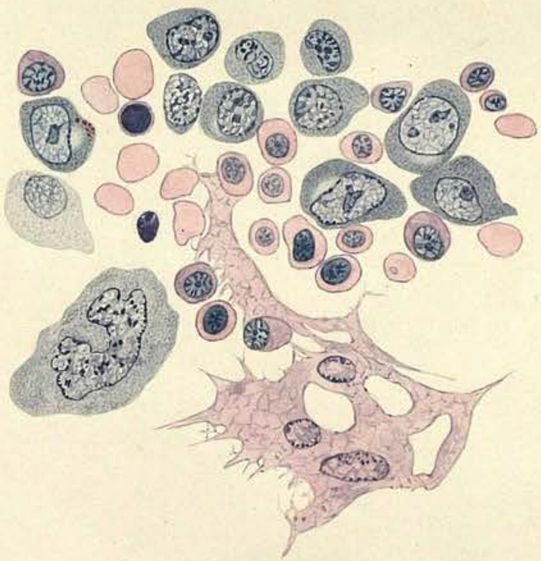


Plate I



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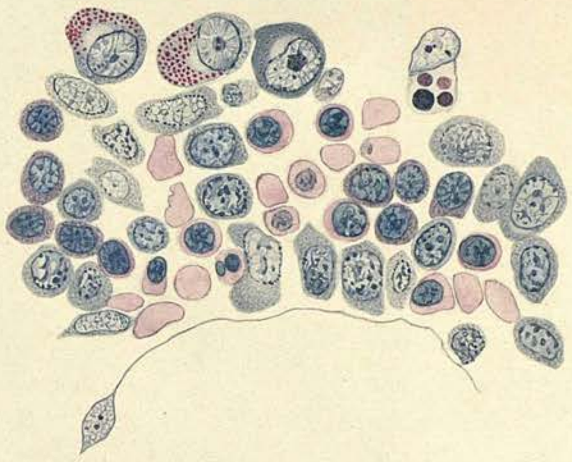

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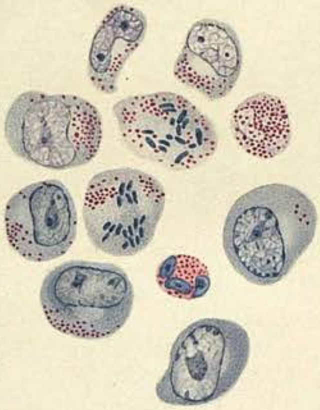

Plate IV